EVOLUTIONARY TRANSFORMATIONS OF THE REPRODUCTIVE SYSTEM IN EUBRACHYURA (CRUSTACEA: DECAPODA)

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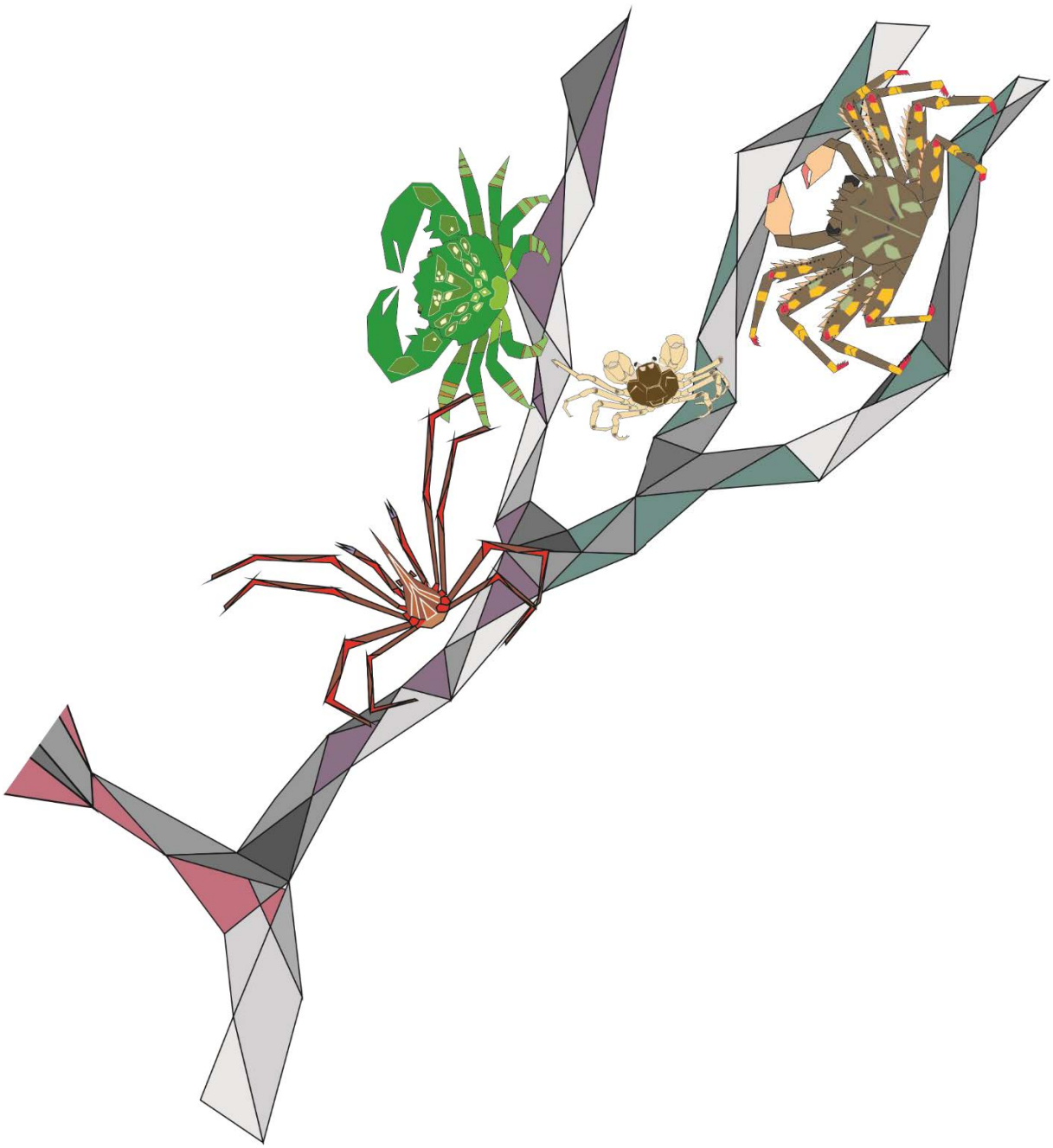


CONTENT

ABSTRACT

The Brachyura comprise approximately 7000 species and belong to the most diverse groups of the decapods. The variability of their morphological traits is reflected in the male copulatory and the female reproductive systems that make them a challenging object of investigation. Numerous studies addressed the brachyuran phylogeny but complete and unambiguous results have yet to be presented. Still, even though it has been controversially debated, some studies still rely on the division of Brachyura into the Podotremata, the Heterotremata and the Thoracotremata (the latter two forming the Eubrachyura) that is based on the position of the male and female gonopores. In this work, the male copulatory and female reproductive systems of four species from three eubrachyuran groups were investigated. For an overview of the structures, the species were photographed with a digital microscope. In the first and the second study, the gonopods were μCT-scanned and 3D-reconstructed to analyse their internal morphology, including the position of muscles and tegumental glands. Additionally, in all studies scanning electron microscopy was used in order to obtain information about the surface structures of the gonopods. All investigations of the female system were conducted using approved histological methods and light microscopy. In the first and second study, this detailed structural analysis was complemented by 3D-reconstruction in order to attain a conception of the positional relationships of the system as a whole within the body.

The first study examines *Mithraculus sculptus* (Lamarck, 1818) and *Stenorhynchus seticornis* (Herbst, 1788) from the heterotreme group Majoidea. Representatives of this group have been intensely studied and hypotheses concerning the structure of the seminal receptacle and a velum as a muscular diaphragm that divides two chambers within it, influenced the conception of eubrachyuran reproduction for decades. The two majoid species were investigated to complement to the growing knowledge of eubrachyuran reproductive systems. Additionally, it is the intent of this study to re-evaluate the interpretation of the velum and to assess its value for future studies. The male gonopods are similar in their general morphology and in the distribution of setae. The tubular first gonopod is longer than the short and stout second gonopod. The general structure of the female reproductive system of *M. sculptus* and *S. seticornis* is the same as in other Eubrachyura. It consists of paired ovaries, oviducts, seminal receptacles and vaginae. In contrast to the prevailing hypothesis, a division of the seminal receptacle into two separate chambers was not observed and the velum might be a character which needs to be re-evaluated.

The second study examines *Percnon gibbesi* (H. Milne Edwards, 1853) from the thoracotreme group Percnidae. The position of the Percnidae is ambiguous. They are probably nested within the polyphyletic Grapsoidea. This study aims to provide comparable characters of the copulatory and reproductive systems of a thoracotreme species in order to find consistencies and differences to heterotreme species. The first gonopod of the male copulatory system is longer than the second

gonopod. Its bent terminal process with a terminal ejaculatory canal opening is a character that is present in other grapsoid species. The female reproductive system reveals a new combination of characters in thoracotremes. The oviduct runs into a separate cuticular duct that transits into the vagina. A direct transition of the oviduct into the seminal receptacle as in other Eubrachyura is absent. Additionally, a bursa that has previously only been described in heterotreme crabs, is connected to the vagina. These morphological characters reveal a higher diversity of thoracotreme reproductive systems than anticipated.

The third study examines the copulatory and reproductive system of *Limnopilos naiyanetri* Chuang and Ng, 1991 from the group Hymenosomatidae. The position of the Hymenosomatidae has been controversially debated for a long time. They have been argued to be a heterotreme group, possibly closely related to the Majoidea. However, the male gonopores have a sternal position, which is a thoracotreme apomorphy. The results are compared with both, data of heterotreme and thoracotreme systems in order to find character states that support an affiliation with either one of the groups. In males, the first gonopod is longer than the second gonopod and the gonopore is sternal. The combination of both characters resembles the thoracotreme condition. In the female reproductive system, the seminal receptacle is lined by a mono-layered glandular epithelium and by a very thin cuticle that is continuous with the vagina. Additionally, a bursa is present. Thus, both systems indicate that Hymenosomatidae are most likely part of the Thoracotremata.

The results of these studies are evaluated in comparison with the existing literature in order to define characters of the male copulatory and female reproductive system and discuss their potential for phylogenetic investigations. Additionally, an evolutionary scenario of the transformations of the herein proposed character states of the female reproductive system is discussed. Without additional information from the female reproductive system, the gonopod morphology is valuable to identify species affiliations to certain groups but remains inconclusive for large-scale brachyuran phylogeny. The last decades brought an increasing number of detailed investigations of the eubrachyuran female system. Some of the characters found in these studies can explicitly be assigned to heterotreme or thoracotreme females (for example the pattern and distribution of tissue in the seminal receptacle, the position of the oviduct transition into the seminal receptacle and the shape of the vagina). The proposed scenarios suggest, that some, if not all of these characters probably have evolved multiple times. In future studies, it needs more strong efforts and the utilisation of modern technology and new approaches to promote the knowledge of these diverse, unique and most beautiful structures in order corroborate the assumptions and reasoning of this study.

ZUSAMMENFASSUNG

Die Brachyura umfassen etwa 7000 Arten und bilden eine der vielfältigsten Gruppen innerhalb der Decapoda. Die Variationsbreite ihrer morphologischen Merkmale spiegelt sich in den männlichen Kopulations- und den weiblichen Reproduktionssystemen wider und machen sie zu einem herausfordernden Untersuchungsgegenstand. Obwohl sich zahlreiche Studien mit der Phylogenie der Brachyura befasst haben, fehlen weiterhin vollständige und eindeutige Ergebnisse. Trotz der kontroversen Diskussion der Phylogenie der Brachyura, stützen sich manche Studien weiterhin auf deren Teilung in die Podotremata, die Heterotremata und die Thoracotremata (die beiden letzteren bilden die Eubrachyura), die auf der Position der männlichen und weiblichen Gonoporen basiert. In dieser Arbeit wurden die männlichen Kopulations- und weiblichen Reproduktionssysteme von vier Arten aus drei Gruppen der Eubrachyura untersucht. Für einen Überblick über die Strukturen wurden die Arten mit einem digitalen Mikroskop fotografiert. Zur Analyse ihrer inneren Morphologie, einschließlich der Position der Muskeln und der Tegumentaldrüsen, wurden die Gonopoden in der ersten und zweiten Studie µCT-gescannt und 3D-rekonstruiert. Zusätzlich wurden in allen Studien rasterelektronenmikroskopische Untersuchungen angewendet, um Informationen über die Oberflächenstrukturen der Gonopoden zu erhalten. Alle Untersuchungen des weiblichen Systems wurden mit bewährten histologischen Methoden und Lichtmikroskopie durchgeführt. In der ersten und zweiten Studie wurde diese detaillierte Strukturanalyse durch 3D-Rekonstruktion ergänzt, um eine Vorstellung von den Lagebeziehungen des Gesamtsystems im Körper zu erhalten.

Die erste Studie untersucht *Mithraculus sculptus* (Lamarck, 1818) und *Stenorhynchus seticornis* (Herbst, 1788) aus der heterotremen Gruppe Majoidea. Vertreter dieser Gruppe wurden intensiv untersucht und Hypothesen über die Struktur des Receptaculum seminis und eines Velums in Form einer muskulären Scheidewand, welches dieses in zwei Kammern teilt, beeinflusst Konzepte zur Reproduktion der Eubrachyura seit Jahrzehnten. Die zwei majoiden Arten wurden untersucht, um das wachsende Wissen der eubrachyuren Fortpflanzungssysteme zu ergänzen. Darüber hinaus ist es die Absicht dieser Studie, die Interpretation des Velums neu zu bewerten und für zukünftige Studien einzuschätzen. Die männlichen Gonopoden sind in ihrer Morphologie und in der Verteilung der Setae ähnlich. Der röhrenförmige erste Gonopod ist länger als der kurze und stämmige zweite Gonopod. Die allgemeine Struktur des weiblichen Reproduktionssystems von *M. sculptus* und *S. seticornis* stimmt mit der in anderen Eubrachyura überein. Es besteht aus paarigen Ovarien, Ovidukten, Receptacula seminis und Vaginen. Im Gegensatz zu der vorherrschenden Hypothese, wurde eine Teilung des Receptaculum seminis in zwei getrennte Kammern nicht beobachtet, wodurch eine Neubewertung des Velums als Merkmal erforderlich wird.

Die zweite Studie untersucht *Percnon gibbesi* (H. Milne Edwards, 1853) aus der thoracotremen Gruppe Percnidae. Deren phylogenetische Position innerhalb der Thoracotremata ist nicht geklärt, liegt aber wahrscheinlich innerhalb der polyphyletischen Graposidea. Diese Studie soll vergleichbare Merkmale der Kopulations- und Reproduktionssysteme einer thoracotremen-Art diskutieren, um Übereinstimmungen und Unterschiede zu heterotremen Arten zu finden. Der erste Gonopod des männlichen Kopulationssystems ist länger als der zweite Gonopod. Sein gebogener terminaler Prozess mit einer terminalen Öffnung des Ejakulationskanals ist auch in anderen Arten der Grapsoidea vorhanden. Das weibliche Fortpflanzungssystem zeigt eine neue Merkmalskombination innerhalb der Thoracotremata. Der Ovidukt mündet in einen separaten kutikulären Gang, der in die Vagina übergeht. Ein, wie in anderen Eubrachyura üblich, direkter Übergang des Ovidukts in das Receptaculum seminis ist nicht vorhanden. Außerdem ist eine Bursa, welche bisher nur bei heterotremen Krabben beschrieben wurde, mit der Vagina verbunden. Diese morphologischen Merkmale zeigen eine größere Vielfalt von thoracotremen Reproduktionssystemen als erwartet.
Die dritte Studie untersucht das Kopulations - und Reproduktionssystem von *Limnopilos naiyanetri* Chuang und Ng, 1991 aus der Gruppe Hymenosomatidae. Die Position der Hymenosomatidae wird seit langem kontrovers diskutiert. Es wurde argumentiert, sie sei eine heterotreme Gruppe, die möglicherweise eng mit den Majoidea verwandt ist. Die männlichen Gonoporen sind jedoch sternal positioniert, welches eine thoracotreme Apomorphie darstellt. Die Ergebnisse werden sowohl mit Daten von heterotremen als auch von thoracotremen Systemen verglichen, um Merkmalszustände zu finden, die eine Zugehörigkeit zu einer der Gruppen unterstützen. Bei den Männchen ist der erste Gonopod länger als der zweite Gonopod und die Gonopore ist sternal. Die Kombination beider Merkmale weist auf den thoracotremen Zustand hin. Im weiblichen Fortpflanzungssystem ist das Receptaculum seminis von einem einlagigen glandulären Epithel und einer sehr dünnen Kutikula, welche in die Vagina übergeht, ausgekleidet. Zusätzlich ist eine Bursa vorhanden. So weisen beide Systeme darauf hin, dass Hymenosomatidae höchstwahrscheinlich Teil der Thoracotremata sind.
Die Ergebnisse dieser Studien wurden in Zusammenhang mit der vorhandenen Literatur interpretiert, um Merkmale des männlichen Kopulations- und weiblichen Reproduktionssystems zu definieren und deren Potenzial für phylogenetische Untersuchungen zu diskutieren. Außerdem wird ein evolutives Scenario bezüglich der Transformation der hier vorgeschlagenen Merkmalszustände des weiblichen Reproduktionssystems diskutiert. Die Gonopoden sind wertvoll, um Artenzugehörigkeiten zu Brachyurengruppen zu identifizieren, sind aber für Untersuchungen großskaliger Brachyurenphylogenie ungeeignet. In den letzten Jahrzehnten wurden vermehrt detaillierte Untersuchungen des weiblichen Reproduktionssystems der Eubrachyura durchgeführt. Einige der Merkmale, welche in diesen Studien dargestellt wurden, können ausschließlich heterotremen oder thoracotremen Weibchen zugeordnet werden (z.B. das Muster und die Verteilung von Gewebe im

Receptaculum seminis, die Position der Oviduktmündung in das Receptaculum seminis und die Form der Vagina). Die vorgeschlagenen Szenarien deuten darauf hin, dass einige, wenn nicht alle dieser Charaktere mehrfach entstanden sind.

In zukünftigen Studien braucht es große Anstrengungen und den Einsatz moderner Technologien und neuer Ansätze, um das Wissen über diese vielfältigen, einzigartigen und schönsten Strukturen voran zu treiben, um die Annahmen und Begründungen dieser Studie zu untermauern.

1 | INTRODUCTION

1.1 | THE BRACHYURA

Crabs (Brachyura) are among the most diverse and intensely studied groups of decapods (Ng et al., 2008; Davie et al., 2015b). Representatives of the Brachyura were able to colonise almost every marine habitat but also some terrestrial environments and their impressive evolutionary radiation resulted in a greater number of species (ca. 7000) than in any other decapod group (Ng et al., 2008; Davie et al., 2015b). The morphology of the Brachyura is enormously diverse. As a derived condition from a long-tailed, lobster-like ancestral body shape, a process called carcinisation caused the disparate habitus of brachyurans (Scholtz, 2014; Keiler et al., 2017). It is amongst others represented by their great size range, an enormously variable body shape and in some cases the dorsal flexion of the last pair of legs in order to hold, for example, sponges or shells for camouflage reasons (Davie et al., 2015a). But most importantly, carcinisation resulted in a reduced pleon that is tightly folded under the thorax, which was interpreted as a key factor for the success of the Brachyura (Ng et al., 2008; Davie et al., 2015a). However, Scholtz (2014) pointed out that the crab-shape cannot be considered advantageous to other body organisations because evolutionary success is not only a matter of species number but must be considered from an ecological and functional context as well. Still, the pleon in the Brachyura is no longer used for locomotion and is sexually dimorphic. In males, it is slender and covers the paired gonopods (first and second pleopods), whereas in females, it is broad and supports the protection of reproductive organs as well as the nursery of fertilised eggs. This brings advantages, including the ability to inhabit the great variety of ecological niches as it made the brachyuran reproduction less dependent on aquatic habitats (even though at a certain stage the larvae must enter water for some time) (McLay & Becker, 2015).

1.1.1 | OBJECT OF INVESTIGATION

The reproductive systems of male and female Brachyura have been subject of many studies throughout the last decades (Spalding, 1942; Cronin, 1947; Hartnoll, 1968; Beninger et al., 1988; Diesel, 1989; Beninger et al., 1991; Diesel, 1991; Minagawa et al., 1994; Lanteigne et al., 1996; Sainte-Marie & Sainte-Marie, 1998; Brandis et al., 1999; López-Greco et al., 2009; Lautenschlager et al., 2010; Becker et al., 2011; Becker et al., 2012; Pardo et al., 2013; Zara et al., 2014; Ewers-Saucedo et al., 2015; Vehof et al., 2016; Becker & Scholtz, 2017; de Souza et al., 2017; Farias et al., 2017; Kienbaum et al., 2017; Pardo et al., 2017; Vehof et al., 2017; Antunes et al., 2018; Ocampo et al., 2018; Vehof et al., 2018; Kienbaum et al., 2018a; Kienbaum et al., 2018b). The diversity and divergence of the reproductive characters make them an intriguing object of investigation. Studies of these structures focused on ecological aspects such as seasonal changes in combination with reproductive strategies

that are important for fishing economy and conservation strategies of economically important species (Armstrong, 1988; Bawab & El-Sherief, 1988; Sainte-Marie & Lovrich, 1994; Hines et al., 2003). They have also been investigated in various other contexts, such as, copulatory behaviour, sperm competition or cryptic female choice (Diesel, 1986, 1991; Sainte-Marie & Lovrich, 1994; Jensen et al., 1996; Jivoff, 1997; Cobo, 2002; Sal Moyano & Gavio, 2012; Castilho-Westphal et al., 2013; Klaus et al., 2014). Studies of eubrachyuran females also focused conceptually on morphological aspects of the internal initiation of fertilisation and sperm storage through moult that are unique within crustacean reproduction (McLay & López-Greco, 2011). Additionally, brachyuran reproductive systems have also come into the focus of phylogenetic studies (Guinot, 1977; Jamieson et al., 1995; von Sternberg & Cumberlidge, 2001).

Today's availability of modern non-invasive techniques, such as, μCT-scans and 3D-reconstruction and their use in combination with well-established methods, such as, scanning electron microscopy and histology facilitate the study of the brachyuran reproductive system in greater morphological detail than previously possible. The data obtained by such a multi-methodological approach yields a thorough understanding of these systems and promises to help contemplating about the value of these structures for phylogenetic studies.

THE MALE COPULATORY SYSTEM

The transformation of the paired first and second pleopods into gonopods in male brachyurans resulted in a unique copulatory system (Fig. 1.3). The paired first and second gonopods (G1, G2) enable the precise placement of sperm within the female system.

Depending on the species investigated, the G1 consists of three or two podomeres (Beninger et al., 1991; Tsuchida & Fujikura, 2000; Benhalima & Moriyasu, 2001; Sal Moyano et al., 2011; Vallina et al., 2014). Although some studies suggest that the distal podomere represents the endopod (Shen, 1935; McLay & Becker, 2015), it remains speculative. Therefore, protopod or endopod will not be used. Instead, proximal, middle or distal podomere adequately describe these structures. The elongation and tubulation of the distal podomere of the G1 creates an ejaculatory canal with one proximal opening and one at the tip.

There are only few data on the G2 but it also seems to comprise three or two podomeres (Beninger et al., 1988; Tsuchida & Fujikura, 2000; Benhalima & Moriyasu, 2001). A tendency towards a reduction of these three podomeres in species deeply nested within the brachyuran tree has been suggested (Guinot et al., 2013; McLay & Becker, 2015). The G2 is in most cases inserted into the proximal opening of the distal podomere of the G1 and comes to lie within the ejaculatory canal (Fig. 1.3). It can be short and is in this case positioned within the proximal area of the distal podomere of the G1 (Fig. 1.3c). Alternatively, it can have an elongated distal podomere, which runs through the

whole length of the distal podomere of the G1 and protrudes from the distal opening of the ejaculatory canal (Fig. 1.3d). In the first case, the G1 is the actual intromittent organ, whereas this function is fulfilled by the G2 in the latter. Either way, the G2 assists in the transportation of the sperm, released by the penis into the genital ducts of the female.

Different types of setae and denticles are positioned along both gonopods. They have been reasoned to provide mechanosensory information during copulation (Beninger et al., 1991; Minagawa, 1993), rupture spermatophores before they enter the female system (Rorandelli et al., 2008) or act as filter structures to prevent particles from entering the ejaculatory canal (Kienbaum et al., 2018a: chapter 3).

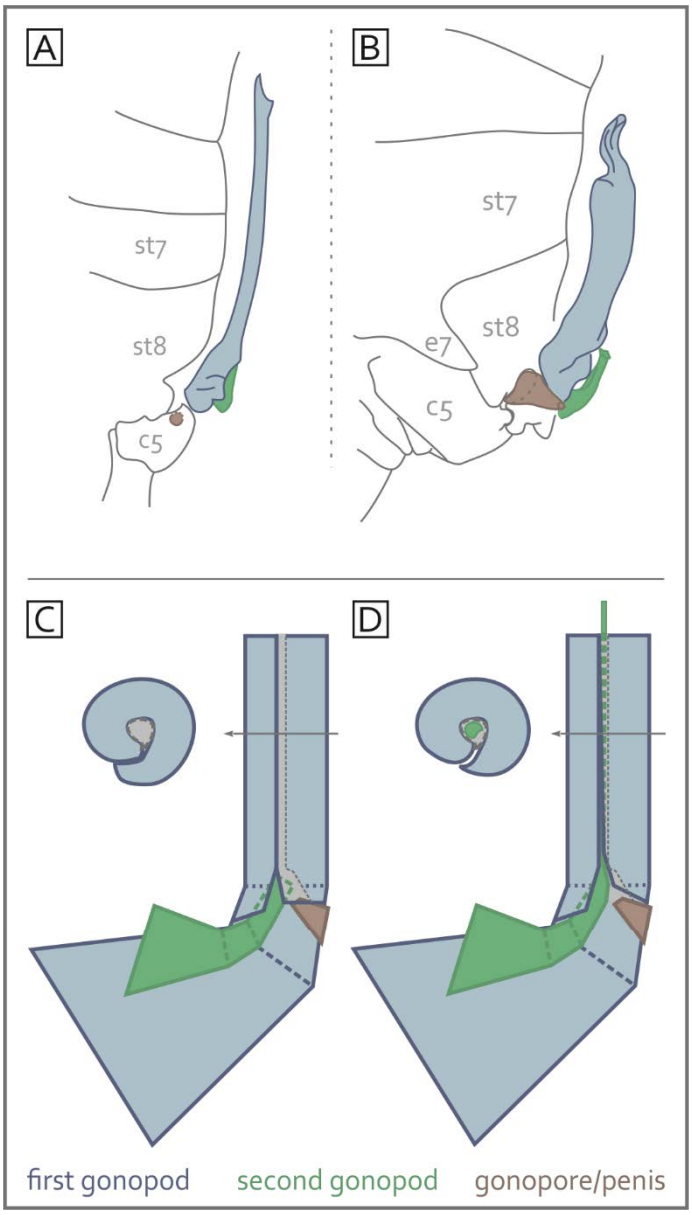


Fig. 1.3 Schematic drawing of the position of gonopods and gonopore /penis. (**A**) Heterotreme males: *Mithraculus sculptus*, right side of the sternum, view from ventral. The gonopore is situated on the coxa of the 5th pereiopod. The penis is not shown. The short G2 is inserted into the proximal opening of the ejaculatory canal of the G1. (**B**) Thoracotreme males: *Percnon gibbesi*, right side of the sternum, view from ventral. The gonopore is situated on the 8th sternite. The penis and the short G2 have been extracted from the G1 when the specimen was prepared for the investigation. (**C**) Relation of gonopods in species with a short G2, that is positioned in the proximal opening of the ejaculatory canal (grey) of the G1. (**D**) Relation of gonopods in species with a long G2, that is positioned in the ejaculatory canal (grey) of the G1.
Abbreviations: c5 – coxa of 5th pereiopod, e7 – episternite 7, st7/st8 – sternite 7/8

The penis is the external elongation of the ejaculatory duct of the male reproductive system. It is a muscular duct, protruding from the gonopore (Guinot et al., 2013). During the evolution of the Brachyura, the position of the male gonopores changed. Being situated at the basis of the fifth walking leg in all podotreme groups and in Heterotremata, they have been relocated to the 8th sternite in Thoracotremata (see Guinot et al., 2013 for a thorough investigation on brachyuran gonopores) (Fig. 1.3).

Since the external morphology and internal anatomy of the male copulatory system has been suggested to bear important information for phylogenetic studies (Bauer, 1986; Beninger & Larocque, 1998; von Sternberg et al., 1999), it will be in the focus of the here presented work. By contrast, the internal male reproductive system will not be discussed.

THE FEMALE REPRODUCTIVE SYSTEM

Eubrachyuran females show a very complex reproductive system. Morphological changes did not only affect the location of the genital duct openings but also whole organ systems and new types of tissues that have evolved. The female system is paired and can be divided into the ovary, the oviduct, the seminal receptacle and the vagina (from inside to outside, Fig. 1.4).

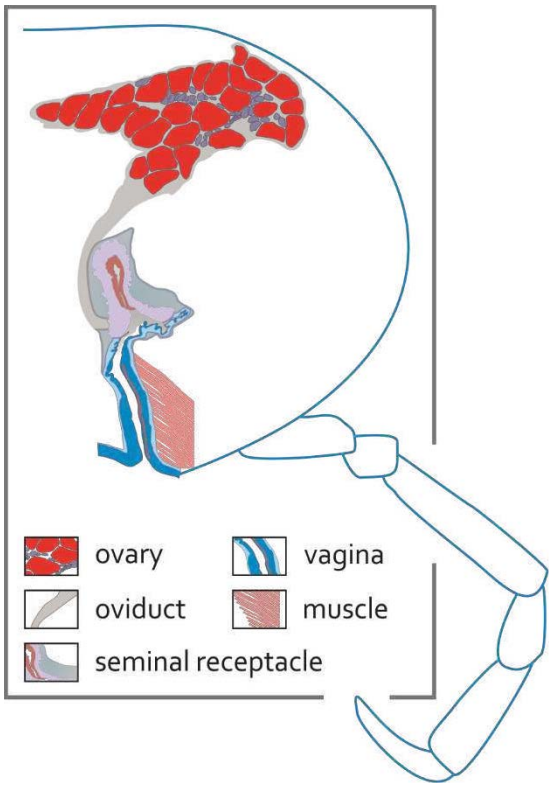


Fig. 1.4 Schematic drawing of the reproductive system of eubrachyuran females. Within the ovaries, early oocytes (purple) develop into mature oocytes (red). During oviposition, an open passage forms between the oviduct (apricot) and the multi-layered secretory tissue of the seminal receptacle (purple, blue). Mature oocytes are transported through the oviduct into the seminal receptacle. The oocytes meet the sperm within the seminal receptacle and fertilisation is initiated internally. The oocytes are then transported through the vagina (blue) towards the gonopore on the 6th sternite. Musculature that connects the inner vagina wall (crescent-shaped type of vagina) to the sternum, facilitates the enlargement of the vagina lumen during oviposition. From here the female carries, grooms and ventilates the fertilised eggs on the broad pleon (maternal brood care).

The ovaries are H-shaped and run as two strands along the length of each side of the body. Both strands are connected through a bridge ventral to the heart (de Souza & Silva, 2009). The oocytes originate within the germinative zone of the ovaries. Afterwards they develop through different stages within the maturation zones. Mature oocytes are transported into the oviduct. As an extension of the ovaries, the oviduct connects them with the seminal receptacle (Kienbaum et al., 2017: chapter 2). The capability to store sperm even beyond the next moult is given by different tissues that show secretory activity and line the seminal receptacle. The latter has a sac-like appearance that can expand, depending on the amount of sperm stored within. The oocytes meet the spermatozoa within the seminal receptacle. Therefore, the initiation of fertilisation is internal in eubrachyuran females (McLay & López-Greco, 2011; but see Vehof et al., 2018).

In addition to the secretory tissue, the seminal receptacle is lined to different degrees by cuticle. This cuticle-lined area is continuous with the vagina. The vagina can be either round or crescent-shaped in cross section (Hartnoll, 1968). Musculature that is connected to the sternum is attached all around the vagina (round shape) or to its concave wall (crescent shape). It probably supports the enlargement of the vagina lumen during copulation and oviposition (Hartnoll, 1968; Kienbaum et al., 2018b: chapter 4). From the seminal receptacle, the oocytes are transported to the outside via the vagina. Both, vagina and its outermost part, the vulva are cuticle-lined and continuous with the 6th sternite, where the gonopore is situated. Female brachyurans carry their eggs under the broad pleon and practice brood care by grooming and ventilating them with oxygen (Baeza & Fernández, 2002; McLay & Becker, 2015).

1.1.2| WHAT WE (DO NOT) KNOW ABOUT THE PHYLOGENY OF EUBRACHYURA

A full review on the existing literature of eubrachyuran phylogeny is beyond the scope of this work. However, some information on the later discussed eubrachyuran groups is indispensable for a better understanding of the status quo.

The taxon Brachyura was established by Latreille in 1802, and since then numerous changes in its nomenclature and classifications have been made (Davie et al., 2015b). Guinot (1977) proposed a system that divided the Brachyura into three groups, based on the position of gonopores (genital openings) (Fig. 1.1). The division into the Podotremata (with coxal gonopores in males and females), the Heterotremata (with coxal gonopores in males and sternal gonopores in females) and the Thoracotremata (with sternal gonopores in males and females) found wide acceptance, as it was convenient and promised to bring clarity into a by then very confusing system. Only shortly after, de Saint-Laurent (1980) united the Heterotremata and Thoracotremata in a monophyletic Eubrachyura based on the position of the female gonopore on the 6th sternite and the presence of a sella turcica (a part of the endophragmal system) in both groups. However, evolutionary processes do not always seem to favour the parsimonious way and phylogenetic relationships have proven to be more complex. Today, with more data at hand, at least podotremes but possibly also heterotremes are no longer considered monophyletic (Spears et al., 1992; Ahyong et al., 2007; Scholtz & McLay, 2009; Karasawa et al., 2011; Shen et al., 2013; Basso et al., 2017).

Unfortunately, there is neither a morphological, nor a molecular phylogenetic analysis that covers all heterotremes, let alone all eubrachyuran groups and presents a good enough resolution that is informative on all levels (Davie et al., 2015b, Fig. 1.2).

For example, Tsang et al. (2014) used an extensive taxon sampling but unfortunately were not able to indisputably resolve the heterotreme groups. The same is true for most molecular studies with a more limited brachyuran taxon sampling (Spears et al., 1992; Ahyong et al., 2007; Bracken et al., 2009; Chu et al., 2009). While there is no doubt about the monophyly of the Thoracotremata (Jamieson et al., 1995; von Sternberg & Cumberlidge, 2001; Tsang et al., 2014), it remains unclear whether the heterotreme brachyurans form a monophyletic group. Those in favour of monophyletic Heterotremata argue on the basis of molecular data (Tsang et al., 2014) but also morphological data such as endosternite characters (sella turcica, Secretan, 1998) and the position of gonopores (Guinot, 1977). Then again molecular data by Bracken et al. (2009), Shen et al. (2013), Yuhui et al. (2017) and Basso et al. (2017) as well as morphological data such as spermatozoal ultrastructure (Jamieson et al., 1995; Jamieson & Tudge, 2000) and foregut ossicles (Brösing et al., 2006) argue against monophyletic heterotremes. More recent studies proposed a sister group relationship of the Potamidae with the Thoracotremata, that would corroborate the heterotreme

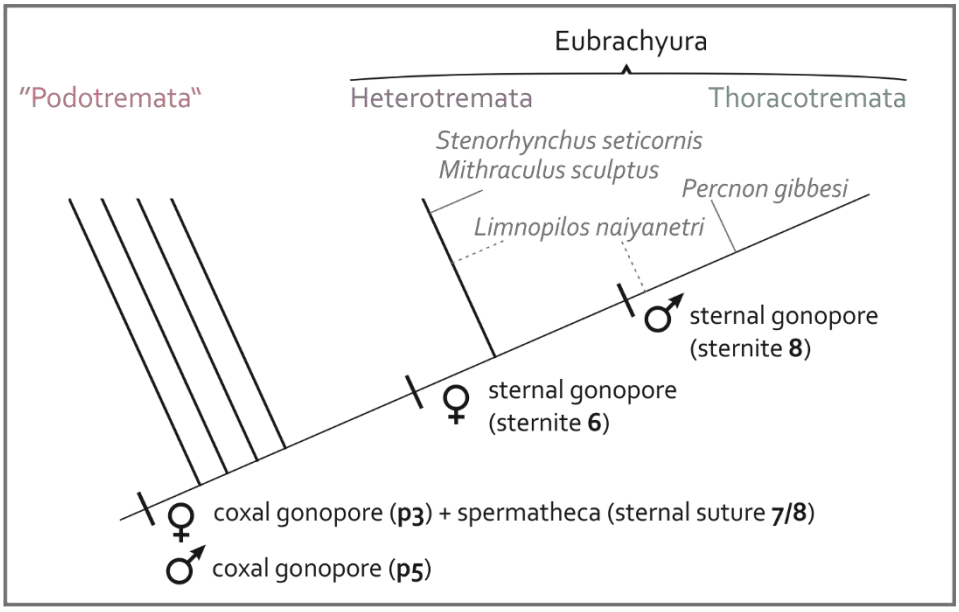
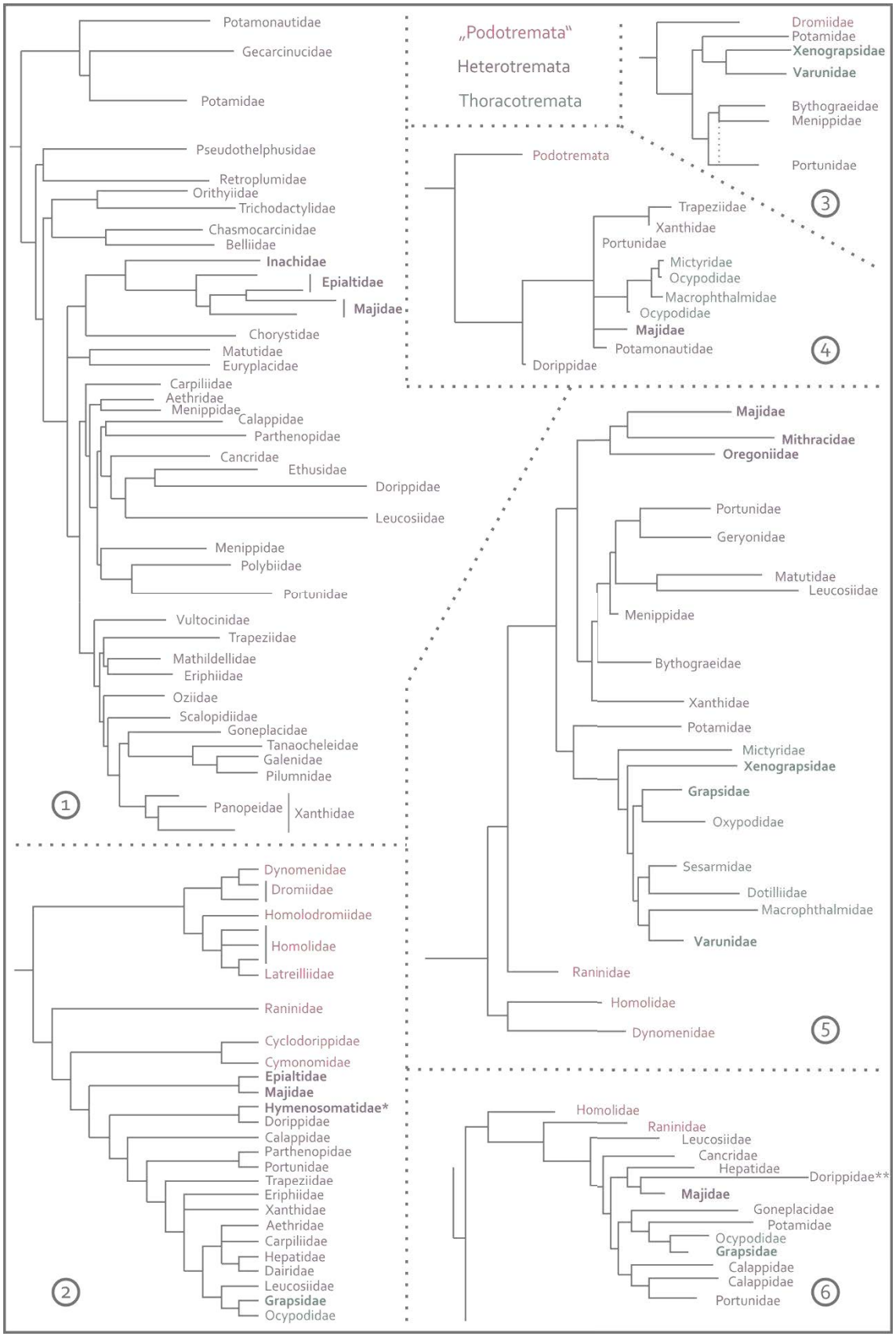


Fig. 1.1 Simplified classification of the Brachyura after Guinot (1977), modified according to the state of knowledge of recent studies (Ahyong et al., 2007; Karasawa et al., 2011; Tsang et al., 2014). The groups were erected based on the position of the male and female gonopores. The "Podotremata" can be assumed to be paraphyletic; * note that the coxal position of the gonopores in podotreme males and females are plesiomorphic characters, while the sternal gonopore in eubrachyuran females and in thoracotreme males are apomorphies of these groups. The Heterotremata and Thoracotremata have been united into the Eubrachyura by Saint Laurent (1980). The species that were investigated in this study are presented in grey: **Majoidea** - *Stenorhynchus seticornis*, *Mithraculus sculptus* (chapter 2); **Grapsoidea** - *Percnon gibbesi* (chapter 3); **Hymenosomatoidea** - *Limnopilos naiyanetri* (chapter 4). The dashed lines indicate the unresolved position of *L. naiyanetri* within the eubrachyuran tree.

Abbreviations: p3/p5 – pereiopod 3/5

Fig. 1.2 The challenging status of brachyuran phylogeny. Different exemplary phylogenetic trees based on molecular (**1-3**, **5-6**) and morphological (**4**) data. (**1**) modified from Tsang et al. (2014) – (only heterotreme groups) (**2**) modified from Ahyong et al. (2007); (**3**) modified from Shen et al. (2013); (**4**) modified from Jamieson et al. (1995); (**5**) modified from Basso et al. (2017); (**6**) modified from Bracken et al. (2009). The groups that are representative for the species investigated in this work (belonging to the Majoidea, the Hymenosomatoidea or the Grapsoidea) in bold letters. * - For the position of Hymenosomatidae that are only shown in (**2**) see also chapter 4. ** - The taxon *Ethusa sp.* investigated by Bracken et al. (2009), was mistakenly assigned to the Dorippidae but is part of the Ethusidae. Note that the poor resolution or support for some nodes of the trees is not shown here. For more detailed information, please see the text and the original studies.


Potamonautidae
Gecarcinucidae
Potamidae
Pseudothelphusidae
Retroplumidae
Orithyiidae
Trichodactylidae
Chasmocarcinidae
Belliidae
Inachidae
Epialtidae
Majidae
Chorystidae
Matutidae
Euryplacidae
Carpiliidae
Aethridae
Menippidae
Calappidae
Parthenopidae
Cancridae
Ethusidae
Dorippidae
Leucosiidae
Menippidae
Polybiidae
Portunidae
Vultocinidae
Trapeziidae
Mathildellidae
Eriphiidae
Oziidae
Scalopidiidae
Goneplacidae
Tanaocheleidae
Galenidae
Pilumnidae
Panopeidae
Xanthidae
1
Dynomenidae
Dromiidae
Homolodromiidae
Homolidae
Latreilliidae
Raninidae
Cyclodorippidae
Cymonomidae
Epialtidae
Majidae
Hymenosomatidae*
Dorippidae
Calappidae
Parthenopidae
Portunidae
Trapeziidae
Eriphiidae
Xanthidae
Aethridae
Carpiliidae
Hepatidae
Dairidae
Leucosiidae
Grapsidae
Ocypodidae
2
„Podotremata"
Heterotremata
Thoracotremata
Podotremata
Trapeziidae
Xanthidae
Portunidae
Mictyridae
Ocypodidae
Macrophthalmidae
Ocypodidae
Majidae
Potamonautidae
Dorippidae
3
4
Dromiidae
Potamidae
Xenograpsidae
Varunidae
Bythograeidae
Menippidae
Portunidae
Majidae
Mithracidae
Oregoniidae
Portunidae
Geryonidae
Matutidae
Leucosiidae
Menippidae
Bythograeidae
Xanthidae
Potamidae
Mictyridae
Xenograpsidae
Grapsidae
Oxypodidae
Sesarmidae
Dotilliidae
Macrophthalmidae
Varunidae
Raninidae
Homolidae
Dynomenidae
5
Homolidae
Raninidae
Leucosiidae
Cancridae
Hepatidae
Dorippidae**
Majidae
Goneplacidae
Potamidae
Ocypodidae
Grapsidae
Calappidae
Calappidae
Portunidae
6

paraphyly (Shen et al., 2013; Basso et al., 2017; Yuhui et al., 2017). Additionally, some eubrachyuran groups might not be monophyletic (Schubart & Reuschel, 2009; Lai et al., 2011, 2014).

Studies of the phylogeny within groups as for example the Majoidea (Hultgren et al., 2009), the Xanthoidea (Karasawa & Schweitzer, 2006; Lai et al., 2011), the Eriphioidea (Lai et al., 2014) or the Grapsoidea (Schubart et al., 2006) present good resolution at lower taxon levels but are lacking support at deeper nodes.

In most studies, the Majoidea represent an early diverging lineage (Spears et al., 1992; Jamieson et al., 1995; Porter et al., 2005) and sometimes even the sister group to all remaining heterotremes (Jamieson et al., 1995; Brösing et al., 2006; Ahyong et al., 2007; Basso et al., 2017). Assuming paraphyletic heterotremes, the Majoidea would very likely represent the sister group to all remaining heterotreme taxa and the Thoracotremata. Additionally, Dorippidae and Ethusidae sometimes form an early diverging lineage (Guinot et al., 2013; Luque, 2015). They are proposed to be early lineages of the eubrachyuran tree, in a sister group relationship with Majoidea (Bracken et al., 2009; Luque et al., 2017). A sister group relationship with Hymenosomatidae as proposed by Ahyong et al. (2007) is only poorly supported and should be rejected.

The position of Leucosiidae is more ambiguous. They also have been placed in a sister group relationship to a monophylum of Dorippidae and Ethusidae, but not close to Majoidea in Tsang et al. (2014). On the other hand, Chu et al. (2009) presented Majoidea, Dorippidae and Leucosiidae in a sister group relationship to Cancridae, but deeply nested within the heterotreme tree. This contrasts to a more recent study by Basso et al. (2017), who proposed a close relationship of Leucosiidae and Matutidae. In Ahyong et al. (2007) the Leucosiidae were even more deeply nested within the eubrachyuran tree as sister group to the thoracotreme taxa included in this analysis. However, the phylogenetic tree of Ahyong et al. (2007) is only poorly supported in most of the nodes, which challenges this interpretation. The internal relationships of the portunoid groups and the position of the Portunoidea is unstable. It has repeatedly been rearranged (Martin & Davis, 2001; Karasawa et al., 2008; Ng et al., 2008; Bracken et al., 2009; Schubart & Reuschel, 2009). Bracken et al. (2009) proposed a sister group relationship of Portunidae and paraphyletic Calappidae, while the data of Tsang et al. (2014) resulted in a sister group relationship of Portunoidea and Menippidae. Unfortunately, this is also only poorly supported and Menippidae are resolved as polyphyletic.

A close relationship of Portunoidea and Majoidea as suggested by McLay & López-Greco (2011) based on mating theories and growth patterns cannot be supported by data from reproductive systems (see discussion).

The Eriphioidea seem to be polyphyletic with its taxa widely spread throughout the heterotreme tree (Lai et al., 2014; Tsang et al., 2014; Davie et al., 2015b). The same seems to be true for the Xanthoidea (Lai et al., 2011) and the groups of Calappoidea, some of which have been placed together with

groups of the Goneplacoidea (Chu et al., 2009; Tsang et al., 2014) or with the Leucosiidae (Basso et al., 2017). The position of the Cancroidea is as unstable as the one of the Portunoidea (Schubart & Reuschel, 2009). However, they seem to be closely associated with Ethusidae, Leucosiidae and Majoidea (Bracken et al., 2009; Chu et al., 2009; Tsang et al., 2014).

While there is no doubt about the monophyly of the Thoracotremata, in the context of large-scale thoracotreme phylogeny its groups are polytomous (Tsang et al., 2014; Basso et al., 2017). At least the Grapsoidea and the Ocypodoidea are polyphyletic with groups intermingling with each other (Tsang et al., 2014).

At the moment the phylogenetic relationships within the Eubrachyura are far from resolved and part of an ongoing debate on how the eubrachyuran groups have evolved.

1.2 | AIMS

The continuing lack of a widely accepted hypothesis on the phylogenetic relationships of the Brachyura clearly demonstrates the need for further phylogenetically informative character complexes. Reproductive systems underlie selective pressures that directly affect the reproductive success of a species and hence its survival. The male copulatory and the female reproductive systems of Eubrachyura present unique character complexes with taxon-specific variations. In order to achieve a denser taxon sampling and to find promising characters that may lead to an understanding of their evolutionary transformation and could potentially be used for phylogenetic purposes, the morphology of the male copulatory and the female reproductive systems of four eubrachyuran species were investigated.

Two species, *Mithraculus sculptus* (Lamarck, 1818) and *Stenorhynchus seticornis* (Herbst, 1788) belong to the Majoidea. This large heterotreme group of approximately 800 species probably diverged early within the eubrachyuran tree (Spears et al., 1992; Jamieson et al., 1995; Porter et al., 2005; De Grave et al., 2009). Their monophyly is widely accepted but internal relationships remain unresolved. Some majoids are commercially important, which led to intense studies of their reproductive characters and resulted in the most thorough investigations of the eubrachyuran reproductive morphology at the time (Beninger et al., 1988; Beninger et al., 1991; Lanteigne et al., 1996; Sainte-Marie & Sainte-Marie, 1998). Amongst them, especially Diesel (1991) influenced conception of these systems for decades.

The third species is the thoracotreme *Percnon gibbesi* (H. Milne Edwards, 1853). It is part of the Percnidae, a probably basal group within the Grapsoidea. However, the Grapsoidea is a thoracotreme group of uncertain phylogenetic position. The monophyly of the Grapsoidea as well as the position of the Percnidae as a possible early diverging lineage within it, have been intensely debated (Schubart et al., 2000; Števčić, 2005; Schubart et al., 2006; Schubart & Cuesta, 2010).

The fourth species is *Limnopilos naiyanetri* Chuang and Ng, 1991. The puzzling group of the Hymenosomatidae consists of more than 100 species that are poorly investigated (Guinot & Richer de Forges, 1997; Ng et al., 2008). The position of the Hymenosomatidae within the eubrachyuran tree is part of an ongoing discussion. Based on several morphological characters and molecular data, the prevailing hypothesis is that they belong to the Heterotremata, possibly in a close relationship to the Majoidea (Chuang & Ng, 1994; Guinot & Richer de Forges, 1997; Ahyong et al., 2007; Guinot et al., 2013). However, the position of the male gonopore is sternal and therefore a thoracotreme character state.

The inconsistencies of some morphological characters in conjunction with their uncertain phylogenetic position make these representatives of three enigmatic eubrachyuran groups intriguing objects of investigation. It is the intent of this study to discuss characters such as musculature and tegumental glands of the male gonopods and embed these into their specific role during copulation. In addition, this work seeks to compile and define possible character complexes of the female reproductive system in order to provide the ability to describe the evolutionary transformations that led to its formation and to discuss their potential for phylogenetic studies.

2 | THE MORPHOLOGY OF THE MALE AND FEMALE REPRODUCTIVE SYSTEM IN TWO SPIDER CRABS (DECAPODA: BRACHYURA: MAJOIDEA) AND THE ISSUE OF THE VELUM IN MAJOID REPRODUCTION.

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ABSTRACT

The reproductive system of spider crabs (Majoidea) has raised considerable interest due to the complexity of female sperm storage organs. In several majoid species, the seminal receptacle has been described as being divided into a dorsal storage chamber and a ventral fertilization chamber separated by a muscular velum. The velum is supposed to control the amount of sperm used for fertilisation and to play an important role in sperm competition. Here, we present a study on the reproductive systems of the two majoid species, *Mithraculus sculptus* (Lamarck, 1818) and *Stenorhynchus seticornis* (Herbst, 1788) using various morphological techniques such as μCT scans and 3D-reconstructions, complemented by paraffin histology. The male gonopods of the herein investigated species are similar in their general morphology and in the presence and distribution of setae. The tubular first gonopod holding the ejaculatory canal is much longer than the short and stout second gonopod, which is supposed to function as a piston in the transport of sperm into the female ducts. The female reproductive system of *M. sculptus* and *S. seticornis* conforms to that of other Eubrachyura in possessing paired ovaries, oviducts, seminal receptacles, vaginae, and vulvae.

Based on our 3D-reconstruction we demonstrate that there is no division of the seminal receptacle into two chambers separated by a velum. In contrast to this, we observed a spatially restricted invagination of the seminal receptacle. A comparison of our data with those of previous studies, allows for the conclusion that the invagination of the seminal receptacle may have been misinterpreted and mistaken for a velum by other authors. Thus, the division of the seminal receptacle into two chambers separated by a velum is a character which needs to be re-evaluated.

KEYWORDS: seminal receptacle, storage chamber, insemination chamber, gonopods, setae, 3D-reconstruction.

2.1 | INTRODUCTION

The Majoidea or spider crabs is a very diverse brachyuran group, comprising more than 800 species, with a worldwide distribution in marine waters (De Grave et al., 2009). It has been proposed to be a basal branching lineage within the Eubrachyura based on morphological (Jamieson et al., 1995) and molecular data (Spears et al., 1992; Porter et al., 2005). However, data concerning the phylogenetic position of the Majoidea within the Eubrachyura is sparse and partially contradictory (Brösing et al., 2006; Tsang et al., 2014). Whilst their monophyly is widely accepted (Hultgren & Stachowicz, 2008; Mahon & Neigel, 2008; Tsang et al., 2014), relationships among the majoid groups remain disputed. The only constant is the monophyly of the Oregoniidae and their position as sister group to the remaining Majoidea (see Hultgren et al., 2009 and Guinot et al., 2013 for review). The morphology of brachyuran reproductive systems has been discussed with respect to phylogenetic (Guinot, 1977; McLay & López-Greco, 2011; Guinot et al., 2013; Becker & Scholtz, 2017) and functional analyses (Diesel, 1989; Beninger et al., 1991).

The male copulatory system is a complex arrangement of three paired parts, consisting of the first and second gonopods and the penes. The tubular first gonopod (G1) forms the ejaculatory canal with a basal and distal opening. During copulation, the second gonopod (G2) and the penis are inserted into the G1 through the basal opening. The penis extrudes the sperm into the ejaculatory canal of the G1. It is then pushed by the G2 into the female genital duct (Bauer, 1986). Gonopods contain valuable phylogenetic information(Beninger et al., 1991; Beninger & Larocque, 1998). This is based on their diverse and specific morphology including the patterns of setae in combination with the conservative nature of gonopods (Martin & Abele, 1986; Vallina et al., 2014).

The female reproductive system of eubrachyuran crabs consists of paired ovaries, oviducts, seminal receptacles, vaginae, and vulvae. The seminal receptacles play a major role in eubrachyuran reproduction due to their ability to store sperm from one or more males that can be used to fertilize several consecutive broods (Cheung, 1968).

The reproductive morphology of the Majoidea has been addressed in several studies (Diesel, 1991; Beninger et al., 1993; Lanteigne et al., 1996; Sainte-Marie & Sainte-Marie, 1998; Rotllant et al., 2007; González-Pisani et al., 2011; Antunes et al., 2016). In contrast to other eubrachyurans, a division ofthe seminal receptacle into two distinct chambers has been described in some majoids (Diesel, 1989). According to this view, a muscular diaphragm, the velum, separates the seminal receptacle into a dorsal secretory "storage chamber" filled with sperm and a ventral cuticle "insemination chamber" with the oviduct junction (Diesel, 1989). The putative existence of a velum has stimulated inferences on the reproduction of spider crabs in terms of the location of fertilisation (Diesel, 1991) and sperm competition of eubrachyurans in general (McLay & López-Greco, 2011). To date, it is not clear which majoid groups actually possess a velum and whether all structures referred to as a velum (Sal Moyano et al., 2010; González-Pisani et al., 2011) are really similar to its original description and definition (Diesel, 1989).

In this study, we investigate the male and female reproductive morphology of the majoids *Stenorhynchus seticornis* (Herbst, 1788), belonging to the Inachoididae, and *Mithraculus sculptus* (Lamarck, 1818) of the Majidae. Our main goal is to re-evaluate characters of the female reproductive system described by Diesel (1989, 1990, 1991) in particular the division of the seminal receptacle into two chambers. By means of the latest morphological methods such as μCT-scans and 3D-reconstruction, together with established histological tools, our results reveal that seminal receptacles of both studied species are not divided into two separate chambers and shed doubt on previous interpretations of such a division and the presence of a velum (Diesel, 1989; Sal Moyano et al., 2010; González-Pisani et al., 2011). The present study demonstrates the value of 3D-reconstructions to understand the spatial organization of reproductive systems and the need for further studies in order to re-evaluate the majoid reproductive systems.

2.2 | MATERIALS AND METHODS

2.2.1 | MATERIAL

The specimens of *Stenorhynchus seticornis* and *Mithraculus sculptus* were obtained from commercial vendors (www.shop-meeresaquaristik.de). Three females of each species were used for histology and the 3D-reconstruction of the reproductive organs. All investigated females were mature and two females of *M. sculptus* were ovigerous.

2.2.2 | HISTOLOGY

For the histological analyses, specimens were cold-anaesthetised in a freezer at –18°C for 15 minutes. Whole specimens were preserved either in Bouin's solution or in "Susa Heidenhain"

(MORPHISTO® Evolutionsforschung und Anwendung GmbH, Frankfurt am Main, Germany) for 48–73 hours. For decalcification, specimens were treated in Ethylenediaminetetraacetic acid (EDTA) for 48–72 hours. Specimens were then dehydrated through a series of ethanol solutions and infiltrated (Shandon Hypercenter XP, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and embedded with paraffin. Sections were prepared at 6–8 μm using a rotary microtome Leica RM2255 (Leica Microsystems GmbH, Wetzlar, Germany). All histological sections were stained with the trichromatic Masson-Goldner "light green" (MORPHISTO®, Frankfurt am Main, Germany).

2.2.3 | SCANNING ELECTRON MICROSCOPY (SEM)

The dissected gonopods of two male specimens of each species were cleaned manually after an ultrasonic bath. The first and second gonopods were critical point dried (Bal-Tec CPD 030, Balzers, Liechtenstein) and sputter coated with a gold layer (Bal-Tec SCD 005, Balzers, Liechtenstein). The micrographs were taken using a LEO (Zeiss) 1430 scanning electron microscope (Carl Zeiss Nano Technology Systems GmbH, Oberkochen, Germany) and images were processed with the software COREL DRAW X6 (Corel, Ottawa).

2.2.4 | MICRO-COMPUTER-TOMOGRAPHY (μCT)

One specimen of each species was fixed in "Susa Heidenhain" (MORPHISTO®, Frankfurt am Main, Germany) for 48–73 hours and washed repeatedly in 70% ethanol. After dissecting the pleon together with the attached gonopods, samples were dehydrated through a series of ethanol solutions. For contrast improvement, samples were immersed in a 1% iodine-ethanol solution for 24 h and subsequently critical point dried (Bal-Tec CPD 030, Balzers, Liechtenstein). The samples were X-ray scanned using a Nanotom (Phoenix | x-ray, GE Sensing and Inspection Technologies) high resolution μCT system.

2.2.5 | IMAGE PROCESSING AND 3D-RECONSTRUCTION

The reconstruction of three-dimensional (3D) models was carried out with the AMIRA software (FEI Visualization Sciences Group, Bordeaux). Series of histological sections were photographed using a stereo microscope Axioskop 2 equipped with a camera Axio Cam HRc and processed with the AxioVision 4.3 software (Carl Zeiss Vision GmbH). The images were turned into grey scale and aligned. After the alignment, the 3D reconstruction was carried out by processing image stacks of these virtual sections. The contours of each reproductive structure (differentiated gray scale values) were marked on the virtual cross section with a polygon, and the polygons then used to calculate a surface model of the reproductive system. 3D reconstruction of

the µCT scans was carried out by processing image stacks of virtual sections. These sections were then edited the same way as the aligned histological sections. All images were processed in Corel DRAW X6 and Corel PHOTOPAINT X6 (Corel, Ottawa).

2.3 | RESULTS

2.3.1 | MALE GONOPODS

OVERALL MORPHOLOGY

The seminal duct passes through the coxa of peraeopod 5 and emerges through the sexual opening (gonopore) as a penis. The gonopore lies adjacent to the basal part of the first gonopod (G1). The small penis enters the G1 on the opposite side of the opening for the second gonopod (G2) (Fig. 2.1). In both investigated species the tubular first gonopod is longer than the stout second gonopod (Fig. 2.1).

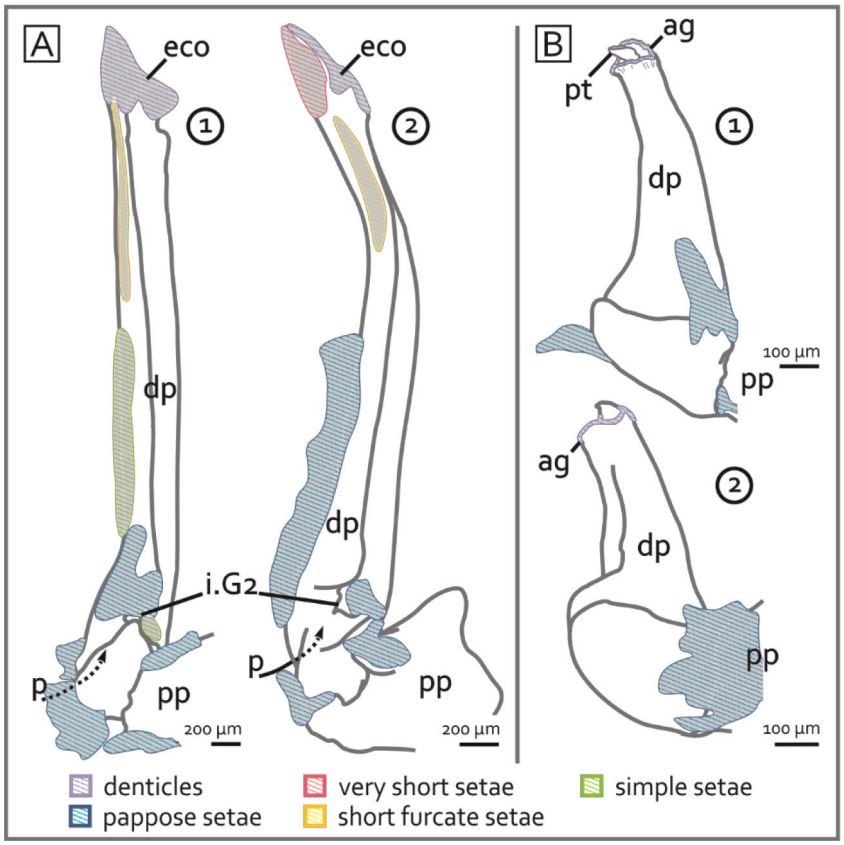


Fig. 2.1 Arrange-ment of different setae types along the gonopods. **A**: First gonopod of (**1**) *Mithraculus sculptus* and (**2**) *Stenorhynchus seticornis*. **B**: Second gonopod of (**1**) *Mithraculus sculptus* and (**2**) *Stenorhynchus seticornis*.
Abbreviations:
ag = apical girdle; dp = distal podomere; eco = ejaculatory canal opening; i.G2 = insertion of G2; p = penis; pp = proximal podo-mere; pt = protuberance.

The G1 (Figs. 2.2, 2.3) is tripartite (Fig. 2.2F). The elongated distal shaft is characterized by the tubular cuticle that forms the ejaculatory canal, whose suture is visible from the outside. The distal, subterminal opening of the ejaculatory canal (eco) is directed medially (Figs. 2.2A,B, 2.3A). The

proximal opening for the second gonopod (G2) is formed between overlapping cuticular folds of the proximal side of the shaft (Figs. 2.2C, 2.3B). Gonopod tegumental glands or rosette glands (rg) are situated proximally within the shaft of the G1, arranged around its basal opening (Fig. 2.2F1–3). Several muscle strands are present in the gonopods connecting the three podomeres (Fig. 2.2F).Within the G1 three muscles are observed (referred to as m1–m3). While the m1 and m2 have only one origin and projection, the m3 has two proximal origins on the ventral and dorsal side of the proximal podomere.

In *Stenorhynchus seticornis* the slightly twisted shaft is bent laterally and tapers distally (Fig. 2.1A2). Its distal end is somewhat bulbous with a pointed tip (Fig. 2.3A).

In *Mithraculus sculptus* the shaft is elongated, straight, and slightly bent in dorso-lateral direction on the first quarter of the basal part. The tip is cone-shaped and elongated on the ventro-lateral side. It is thereby forming a pointed tip with a wide opening of the ejaculatory canal. A small hook like appendix is present on the dorso-medial side below the edge of the tip (Fig. 2.2A,B).

The G2 of both species is tripartite as well. The distal podomere has a compact shaft with a smooth surface and only few folds along its ventro-medial side (Fig. 2.3C). Along the distal part of the shaft the cuticle is very compact and almost completely fills out the entire lumen (Fig. 2.2F4). The G2 has three muscles (referred to as m*1–m*3). One of them (m*1) is running within the distalmost segment only (Fig. 2.2F4).

In both species an apical girdle (sensu BENINGER et al., 1991) surrounds the tip (see dashed lines in Figs. 2.2E, 2.3C). The G2 of *S. seticornis* presents a process at the tip that is pointing dorsally. In *M. sculptus* the tip of the G2 bears a central protuberance (Fig. 2.2E).

SETATION

The types of setae and their distribution along the surface of the gonopods are similar in *S. seticornis* and *M. sculptus* (see Table 2.1 and Fig. 2.1A,B) .

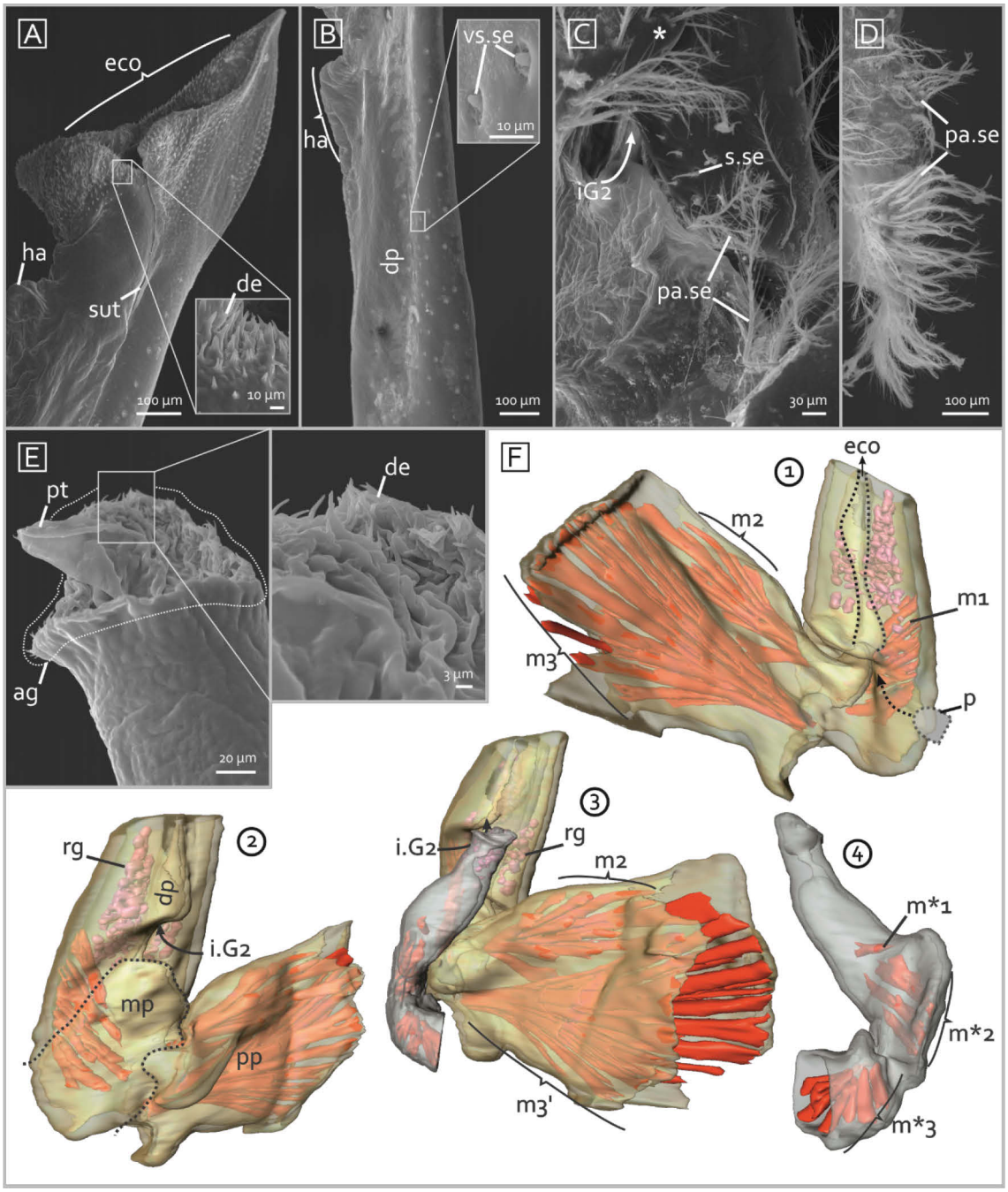


Fig. 2.2 First and second gonopods (G1, G2) of *Mithraculus sculptus*, SEM pictures (A–E) and 3D-reconstruction based on µCT (F). **A**: Tip of G1 with detail of denticles surrounding the ejaculatory canal opening. **B**: Part of G1 with the hook-like appendix. **C**: Proximal part of the folded cuticle (see *) forms the opening for the G2. **D**: Pappose setae along basal 2/3 of dorsolateral edge. **E**: Tip of G2 with the apical girdle (including an enlargement of the denticles) and the protuberance. **F**: 3D-reconstruction of proximal part of G1 (1–3) and G2 (3, 4). Cuticle is presented semi-transparent to allow visibility of the muscle strands and rosette glands within the gonopods. (**1**) Course of ejaculatory canal indicated by black dashed lines. The position of the penis and its insertion into the G1 is indicated by a semi-transparent grey area and arrow. (**2**) All podomeres of G1 can additionally be distinguished through the attachment sites of the muscle strands. Form of middle podomere indicated by dashed line. (**3**) Position of both gonopods. G2 not inserted into G1.

Table 2.1 Location and arrangement of types of setae on first gonopod (G1) and second gonopod (G2) of *Stenorhynchus seticornis* and *Mithraculus sculptus*; + = present; – = absent.

Location / arrangement on gonopod			setae type	*S. seti-cornis*	*M. sculptus*
G1	proximal + middle podomere	• along the dorsal edges	pappose (Fig. 2C-D; Fig. 3D)	+	+
	distal podomere	• surrounding the basal opening for the G1 and penis		+	+
		• along the basal two-thirds of the dorsolateral edge		+	-
		• one row on basal postero-medial edge		+	-
		• along the middle of the dorso- and ventro-lateral area	simple	-	+
		• along the distalmost dorso- and ventro-lateral area	simple, very short (Fig. 2B)	+	+
		• on the dorso-lateral side of the tip	bifurcate, short (Fig. 3A)	+	-
		• one row along one-third of the dorso-lateral side		+	-
		• around the ejaculatory canal opening	denticles (Fig. 3A)	+	+
G2	proximal + middle podomere	• grouped along the distal edge	pappose (Fig. 3C)	+	+
	distal podomere	• grouped along the proximal edge		-	+
		• around the distal apical girdle	denticles (Fig. 2E)	+	+

(**4**) Instead of all other muscle strands both attachment sides of the m*1 are situated within the distal podomere.
Abbreviations: ag - apical girdle; de - denticle; dp - distal podomere; eco - ejaculatory canal opening; ha - hook-like appendix; i.G2 - insertion of G2; m1 - ventral muscle bundle within G1 running within the distal podomeres; m2, m3 - two dorsal muscle bundles within G1 that run from the distal to the proximal podomere; m2', m3' - two ventral muscle bundles within G1 that run from the distal to the proximal podomere; m*1 - muscle bundle running within distal podomere of G2; m*2, m*3 - muscle bundles within G2 running within the distal podomeres and the middle podomere to the proximal podomere, respectively; mp - middle podomere; p - penis; pa.se - pappose setae; pp - proximal podomere; pt - protuberance; rg - rosette glands; s.se - simple setae; sut - suture; vs.se - very short setae; * = cuticle folding that forms part of insertion site of G2.

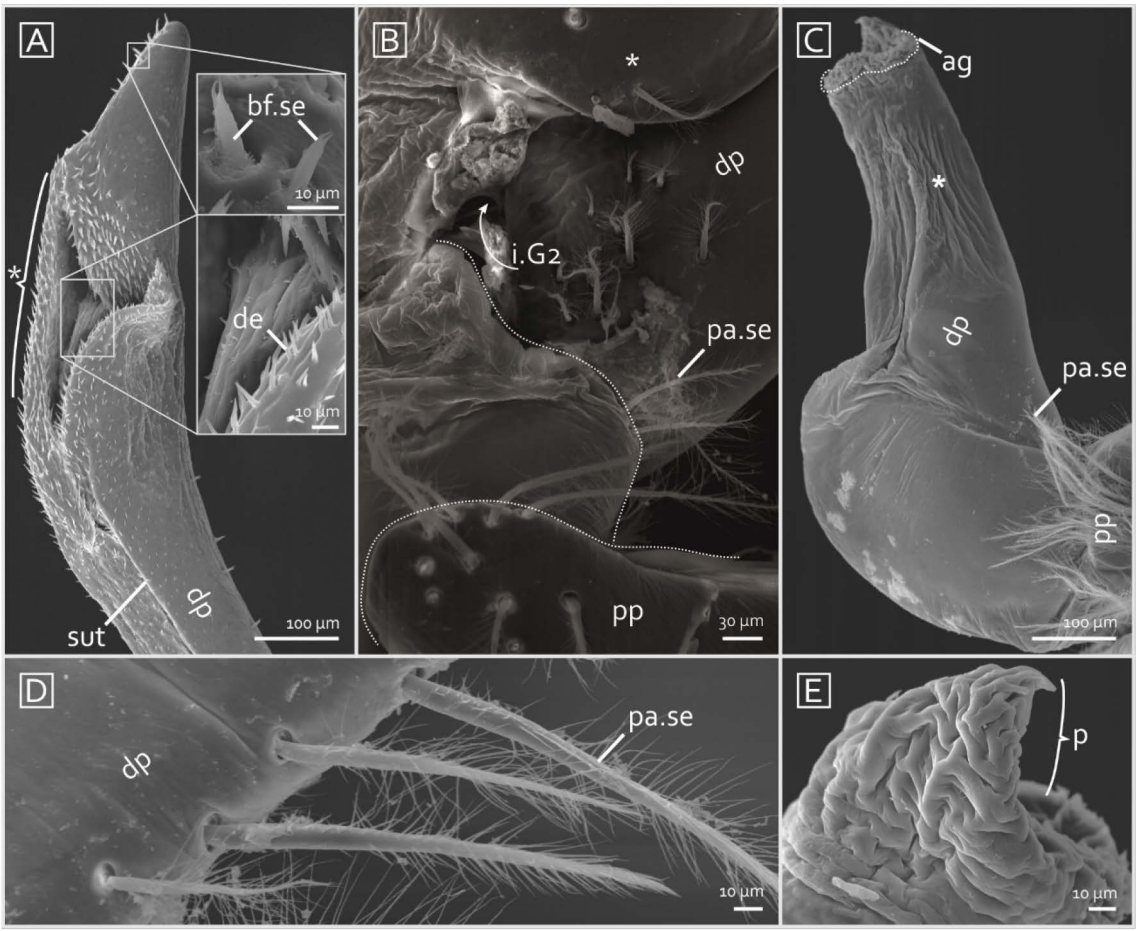


Fig. 2.3 First and second gonopods (G1, G2) of *Stenorhynchus seticornis*, SEM pictures. **A**: Tip of G1 with ejaculatory canal opening (see *) surrounded by denticles (lower enlargement) and bifurcate setae (upper enlargement), suture of folded cuticle clearly visible. **B**: Proximal part of folded cuticle (see *) that forms the opening for G2. Dashed lines mark middle and proximal podomeres. **C**: Overview of G2, apical girdle indicated by dashed line; * indicates area of longitudinal folds along distal podomere. **D**: Pappose setae along edge of G1 distal podomere. **E**: Process at apex of G2.
Abbreviations: ag - apical girdle; am - appendix masculine; bf.se - bifurcate setae; de - denticle; dp - distal podomere; i.G2 - insertion of G2; sut - suture; pa.se - pappose setae; pp - proximal podomere.

2.3.2 | FEMALE REPRODUCTIVE SYSTEM

The females of *Mithraculus sculptus* and *Stenorhynchus seticornis* have very similar reproductive systems that differ only in some small details. Thus, the results presented in the following apply to both species as long as not mentioned separately.

OVARY AND OVIDUCT

The ovaries are paired, elongated organs located dorsally in the cephalothorax with two ovary strands running along each body half as anterior and posterior lobes. The left and right strands

connect ventral to the heart. Whereas those of *M. sculptus* are restricted to the thorax, the posterior ovarian lobes of *S. seticornis* extend into the pleon and are additionally fused posteriorly.

The tissues and cell types that line the ovaries and the oviduct are continuous (Fig. 2.4A–F). Each strand wherein the oocytes develop is highly convoluted and internally lined by a mono-layered epithelium (Fig. 2.4A–C,F). The cells that form the oviduct have a cubic shape with round basally located nuclei, whereas the cells of the ovary are more elongated and have oval nuclei. Both structures are externally coated by a thin layer of connective tissue (Fig. 2.4D,E–F).

Different stages of oocyte development are present within the ovaries (Fig. 2.4B,C). The germinative zones, where oogonia proliferate, can be distinguished from the adjacent maturation zones where the oocytes develop. However, in females with large mature ovaries, the mature oocytes are intermingled with strands of germinative zones. Within the germinative zones, batches of oogonia (10–20 μm) with a low amount of cytoplasm and relatively large nuclei, originate (Fig. 2.4C,F). Previtellogenic oocytes with a larger proportion of cytoplasm and an irregular cell shape (20–120 μm), are also present in the germinative zone (Fig. 2.4C,F). The ovaries contain no oocytes in early vitellogenesis (meaning the stage of the maturing cells, in which the inclusion of yolk has started only recently) but show mature oocytes at sizes of 140–300 μm, completely filled with yolk (Fig. 2.4A–C,E). In several females, oogonia and previtellogenic oocytes are also present within the oviduct close to the connection to the seminal receptacle (Fig. 2.4D,F).

SEMINAL RECEPTACLE

The seminal receptacle (SR) is externally coated by connective tissue. Internally, a dorsal secretory area and a ventral cuticle area can be discriminated (Figs. 2.5A1–4,B, 2.6A1,2,B–C). The tissue of the dorsal area is stratified. Due to an irregular surface, the cells that form the outer cell layer, appear loosely arranged. They are associated with the surrounding connective tissue.

The adjoining proliferative cells form the middle section and an increasing degeneration of cells towards the lumen of the SR results in the release of secretions (Fig. 2.5D). The lumen is filled with sperm masses without any apparent layering or divisions of the sperm masses by sperm gel (Figs. 2.5E,G, 6D).

The oviduct runs into a thickened portion of the secretory tissue (Fig. 2.6C,G), close to the transition into the cuticle epithelium (Fig. 2.4D) and to the adjoining vagina (Fig. 2.5A3). Cuticle folds are present at the transition between the secretory tissue and the cuticle area of the SR (Figs. 2.5F, 2.6C,E). The cuticle that lines the SR ventrally and parts of the dorsal area is formed by a columnar epithelium.

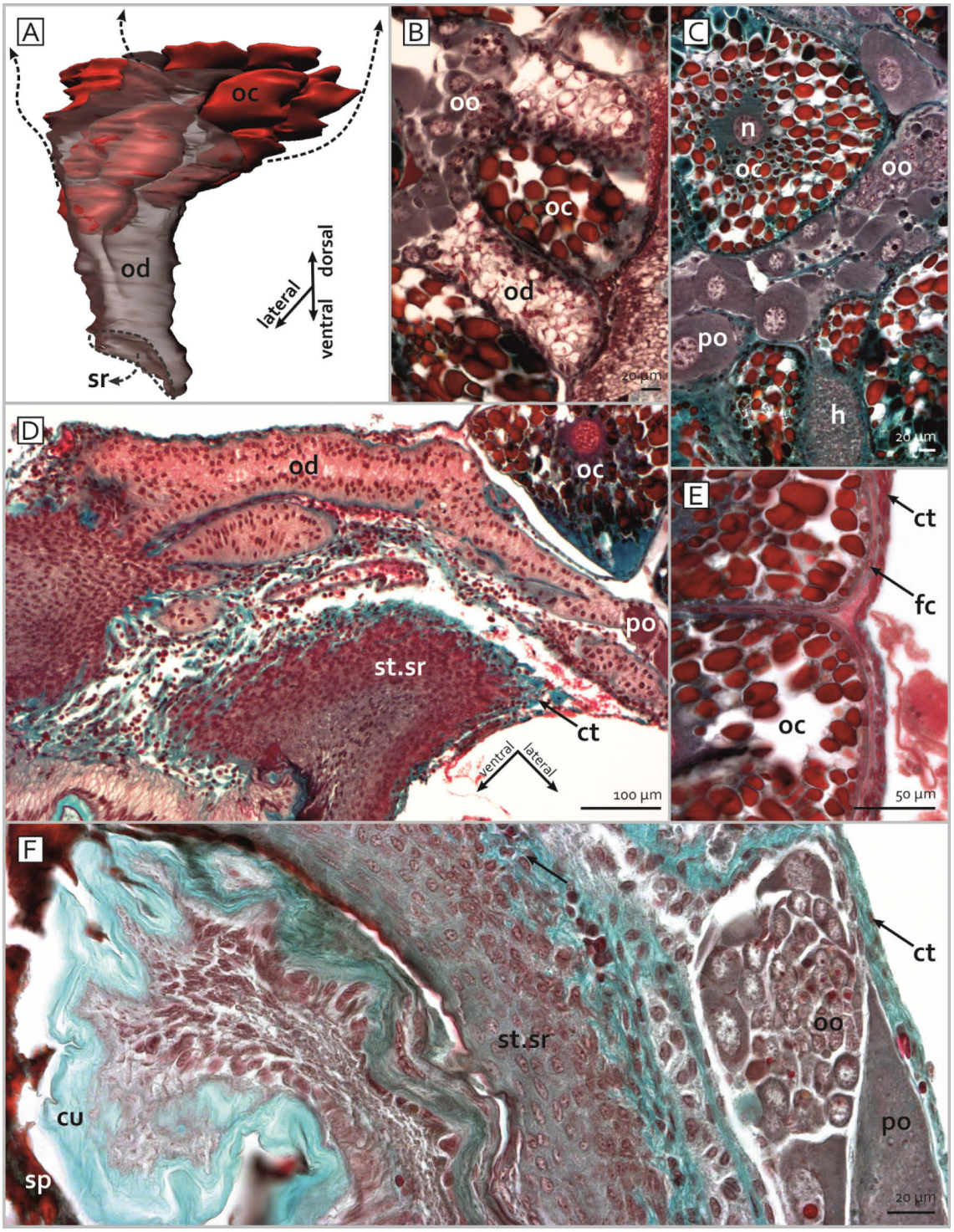


Fig. 2.4 Ovary and oviduct of *Stenorhynchus seticornis* (A,B,E) and *Mithraculus sculptus* (C,D,F). **A**: 3D-reconstruction of oviduct and parts of ovary based on histological sections showing mature oocytes within oviduct. Black dashed arrows = continuation of tissue; dark dashed line and arrow = orifice to seminal receptacle. **B**: Histological section through oviduct within ovary. Oogonia enclosed within oviduct, the mature oocytes arranged adjacent to it. **C**: Cellular organisation within ovary and oviduct. Germinative zones arranged in strands in between the mature oocytes. Some haemal vessels can be found. Notice the central nucleus in the mature oocytes. **D**: Histological longitudinal section of oviduct connecting ovary to seminal receptacle.

In *M. sculptus*, the ventral cuticle-lined part of the SR forms several prominent bulges which protrude into the lumen and partly divide it into different areas (Fig. 2.5A3,4,B). The cuticle occurs also in the dorsal area, where it covers the secretory tissue towards the lumen (Fig. 2.5E). In some sections the seminal receptacle of *S. seticornis* shows a cuticle structure that transforms into a cuticle bulge that protrudes towards the lumen of the ventral area (Fig. 2.6D,F).

THE VAGINA

The cuticle of the SR is continuous with the cuticle of the vagina. In cross sections the vagina is crescent shaped, resembling the "concave type" vagina (sensu Hartnoll, 1968) with the inner wall invaginated into the outer wall occluding the vagina lumen. Two different cuticle layers can be distinguished (see Locke (2001); Fig. 2.7C). The epicuticle faces the vagina lumen and stains red in Masson's trichrome while the procuticle stains blue. The flexible parts of the vagina wall are equipped with musculature and the cuticle herein appears structurally different from the remaining procuticle and stain red in Massons trichrome. In both species investigated, the muscle attachment correlates with the flexible parts of the vagina.

In *M. sculptus* only the inner vagina wall is flexible and connected to the sternum by muscles running diagonally to ventro-lateral (Fig. 2.7A,B,D,E). In *S. seticornis*, also the outer vagina wall appears flexible towards the SR – indicated by red-staining horizontal bands of the procuticle and a muscle attachment (Fig. 2.7E). Those muscles run diagonally ventro-medial to the sternum. In one specimen a sperm plug was found within the vagina lumen (Fig. 2.7F).

E: Magnification of two adjacent mature oocytes within ovary. Notice the "follicle cells" that lie between the oocyte cell membrane and a very fine membrane that is present adjacent to the oocytes. **F**: From right to left: Oogonia and previtellogenic oocytes within oviduct in very close proximity to orifice connecting oviduct and seminal receptacle. The oviduct lies adjacent to the secretory tissue of the seminal receptacle. Towards the seminal receptacle lumen a cuticle bulge covers the secretory tissue. The lumen of the seminal receptacle is filled with sperm mass. Arrows = connective tissue around both structures.

Abbreviations: ct - connective tissue; cu - cuticle; h - haemal vessel; st.sr - secretory tissue of the seminal receptacle; fc - follicle cell; oc - mature oocyte; n - nucleus; oo - oogonia; od - oviduct; po - previtellogenic oocyte; sp - sperm mass; sr - seminal receptacle.

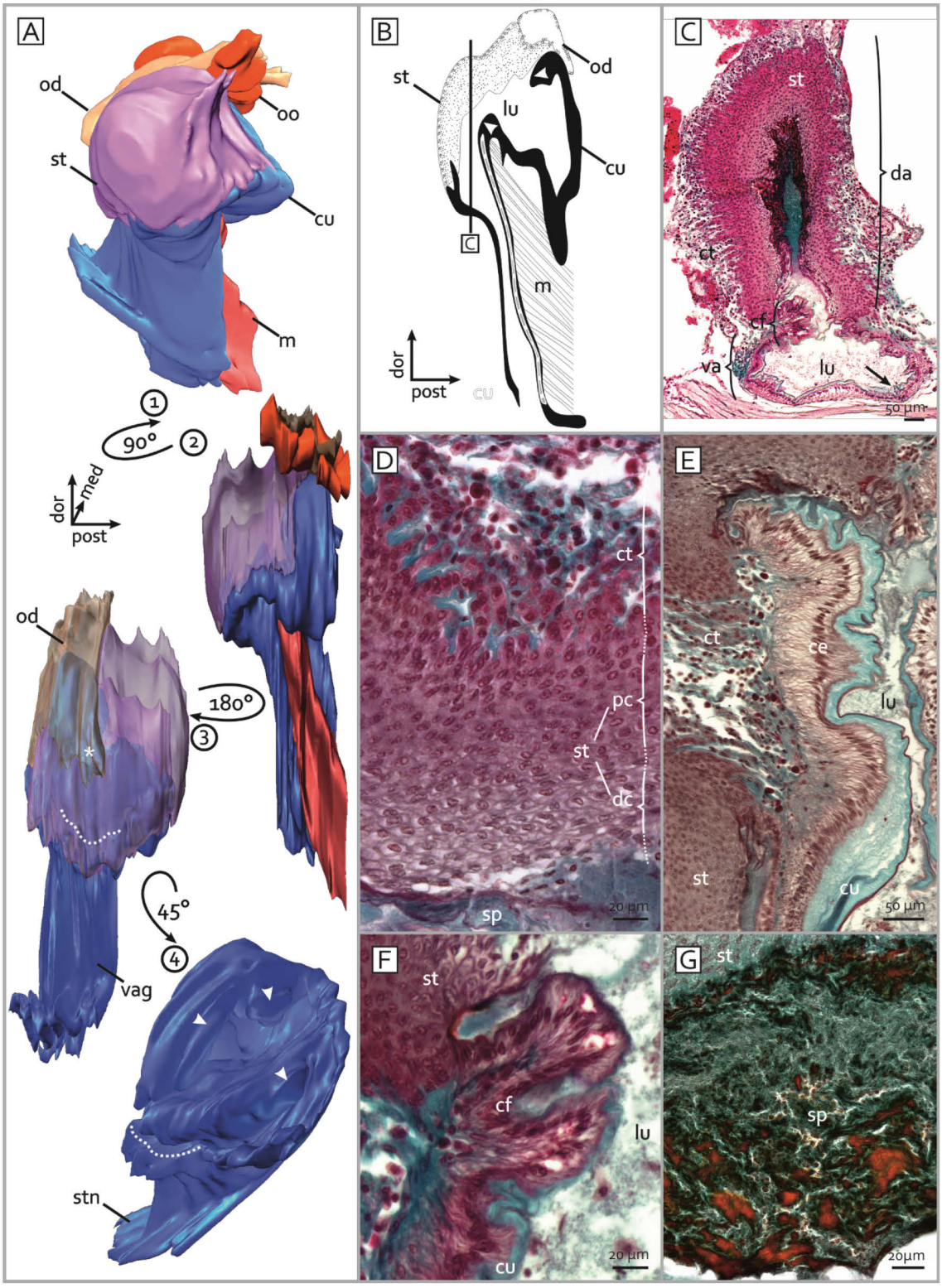
2.4 | DISCUSSION

2.4.1 | THE MALE GONOPODS

OVERALL SHAPE AND FUNCTIONS IN SPERM TRANSFER

The distal segment of the first gonopod (G1) of brachyuran males is folded longitudinally, forming the ejaculatory canal with a basal and a distal opening (Rorandelli et al., 2008; Sal Moyano et al., 2011; Vallina et al., 2014). During copulation, the second gonopod (G2) and the penis are both inserted into the G1 through a basal opening. Together, they form a complex copulatory system to transport the sperm masses into the female seminal receptacles through the vagina during copulation. The relative length of the G1 and G2 is variable among the Brachyura (see McLay & Becker, 2015). A short G2 is characteristic for the Majoidea (Beninger et al., 1991; Diesel, 1991; Neumann, 1996; Rorandelli et al., 2008; Sal Moyano et al., 2011; this investigation) and also present in other eubrachyuran groups (e.g. Ocypodidae: Lautenschlager et al., 2010; Pinnotheridae: Becker et al., 2012). The characters present in the investigated G2 of *Mithraculus sculptus* and *Stenorhynchus seticornis* resemble those of *Chionoecetes opilio* (O. Fabricius, 1788) (Beninger et al., 1991).

Fig. 2.5 Seminal receptacle and associated structures of *Mithraculus sculptus*. **A**: Different perspectives of a 3D-reconstruction of seminal receptacle and parts of oviduct based on histological sections. (**1**) all parts in shaded outlines; (**2**) secretory tissue transparent; (**3**) secretory tissue and oviduct transparent; (**4**) only cuticular parts of reproductive system visible, view from dorso-medial and cuticle slightly tilted in anterior direction. Cuticle of ventral area shows prominent bulges that protrude into the lumen (see arrowheads part 4) and divides it into different compartments. In some areas these bulges cover the secretory tissue towards the lumen (transparent in part 3). The oviduct connection to the seminal receptacle is medio-ventrally (see * in part 3) in very close proximity to the vagina opening (dashed lines in part 3 + 4). **B**: Idealized schematic drawing of seminal receptacle. Notice that the oviduct lies adjacent to the secretory tissue. Flexible parts of inner vagina wall indicated by grey area within the cuticle (arrowheads indicate cuticle bulges). **C**: Histological cross section through seminal receptacle. A dorsal secretory area can be distinguished from a ventral cuticle area. Prominent folds at the transition between the two parts. Arrow = thin cuticle. **D**: Stratified tissue of secretory area of the seminal receptacle. **E**: Cuticle lining of seminal receptacle in dorsal area with stretched columnar epithelium. The cuticle bulges partly cover the secretory tissue that lines the dorsal area. **F**: Prominent cuticle folds at transition between secretory and cuticle area. **G**: The sperm mass within the seminal receptacle is not arranged in layers.
Abbreviations: cb - cuticle bulge; ce - columnar epithelium; ct - connective tissue; cu - cuticle; da - dorsal area; dc - disintegrating cells; dor - dorsal; cf - cuticle folds; st - secretory tissue; lu - lumen; m - muscle; med - medial; oc - oocyte; od - oviduct; pc - proliferating cells; post - posterior; sp - sperm mass; stn - sternum; va - ventral area; vag - vagina.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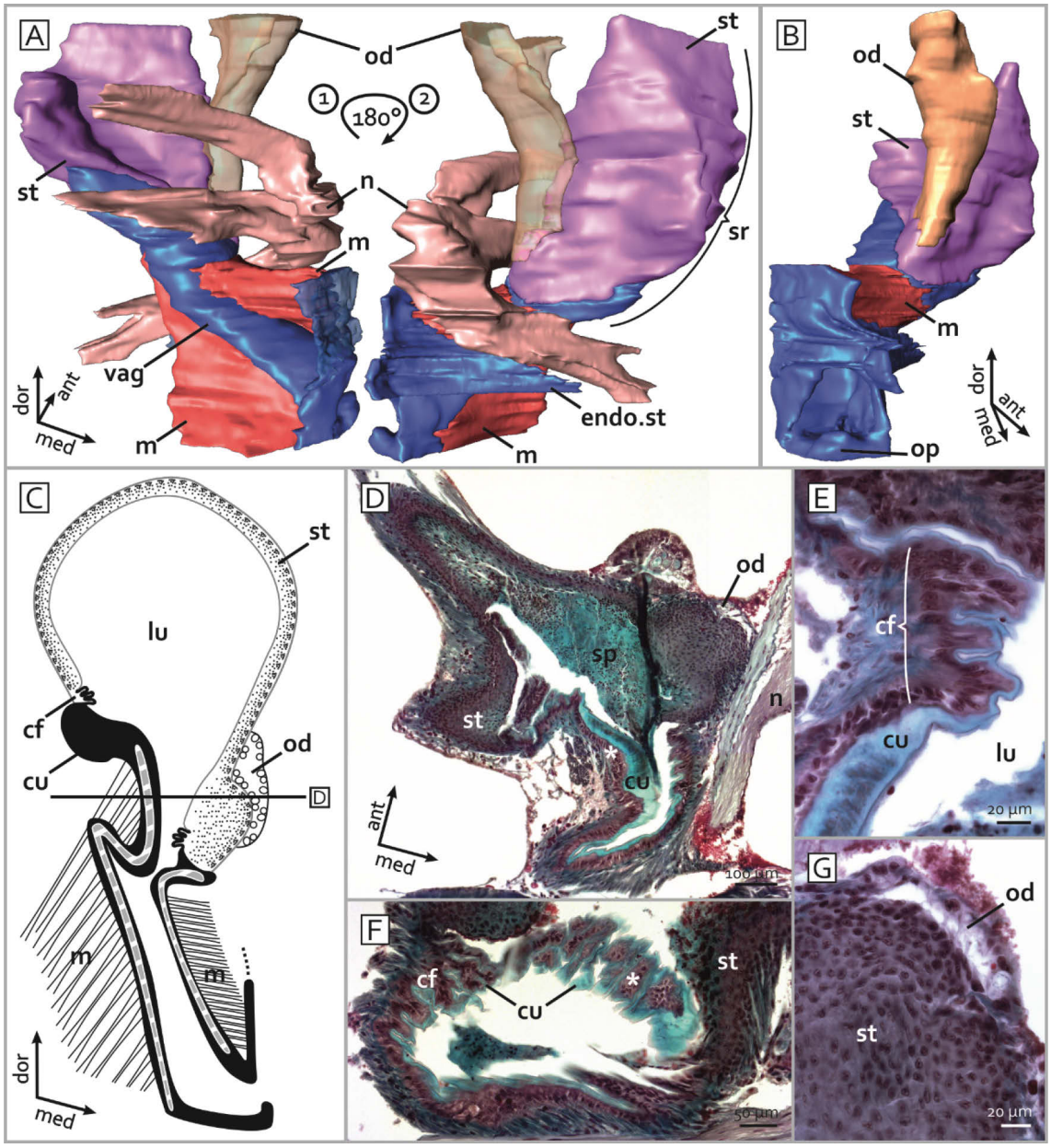

Fig. 2.6 Seminal receptacle and associated structures of *Stenorhynchus seticornis*. **A**: Different perspectives of a 3D-reconstruction of the seminal receptacle and parts of the oviduct based on histological sections. Oviduct and sternum shown transparent. Additionally three nerves coming from ventral nerve cord run along the seminal receptacle in very close proximity. **B**: 3D-reconstruction of seminal receptacle and parts of oviduct based on histological sections. **C**: Idealized schematic drawing of the seminal receptacle in longitudinal section. Secretory tissue forms the dorsal area and is followed ventrally by cuticle. Flexible parts of vagina indicated by grey dashed line within the cuticle. Muscles attached to both sides of vagina. The line indicates approximate position of cross section presented in Fig. 6D. **D**: Cross section through seminal receptacle. The cuticle bulge (see *) transforms into a cuticle structure that can be misinterpreted as a velum (see * in Fig. 6F). At opening of oviduct into seminal receptacle the secretory tissue clusters into a much thicker tissue than anywhere else. **E**: Histological section of folds appearing on transition between secretory and cuticle areas. **F**: Cuticle structure that could be misinterpreted as a velum (see *). **G**: Transition of oviduct into secretory tissue of seminal receptacle.

Even though observations of copulations are rare and data concerning the actual movement of gonopods are lacking, certain hypotheses about the transport of sperm have been developed (Beninger et al., 1991; Becker et al., 2012). In a system with a short G2, the G1 is the actual sperm conduit that interacts with the vagina while the G2 is supposed to have an accessory function by moving the sperm distally within the ejaculatory canal (Beninger et al., 1991). The narrow ejaculatory canal allows only minor movements of the G2 within the G1. With respect to the compact cuticle at the distal part of the G2, it seems unlikely that the G2 can be stretched significantly but it may however act like a seal, as has also been suggested by Beninger et al. (1991). The cuticle folds on its surface (Fig. 3C) might allow the seal to be broken by muscular contractions.

SETATION AND DENTATION

The arrangement of the various setal types along the G1 and G2 of the species investigated is widely consistent with data from other majoid studies (see Table 1). The presence of pappose setae (Figs. 2C,D, 3B–D) at the proximal parts of the gonopods has also been described for *C. opilio* (Beninger et al., 1991) and for *Libinia spinosa* Guérin, 1832 (Sal Moyano et al., 2011). Furthermore, the denticles located on the gonopod tips are present in many other majoid males (Beninger et al., 1991; Diesel, 1991; Neumann, 1996; Sal Moyano et al., 2011) and have been regarded as homologous structures (Beninger et al., 1991).

The denticles surround the distal opening of the ejaculatory canal of the G1 (Fig. 2A,E) and are supposed to rupture spermatophores during sperm transfer (Beninger et al., 1991; Neumann, 1996; Rorandelli et al., 2008). The observation of intact spermatophores in *S. seticornis* by Antunes et al. (2016) and *C. opilio* by Sainte-Marie et al. (2000) however contradicts this assumption.

Abbreviations: ant = anterior; cf = cuticle folds; cu = cuticle; dor = dorsal; endo.st = endo sternit; st = secretory tissue; lu = lumen; med = medial; m = muscle; n = nerve; op = operculum; od = oviduct; sp = sperm mass; vag = vagina; * = cuticle strap that dents into the lumen (compare Fig. 6F and D)

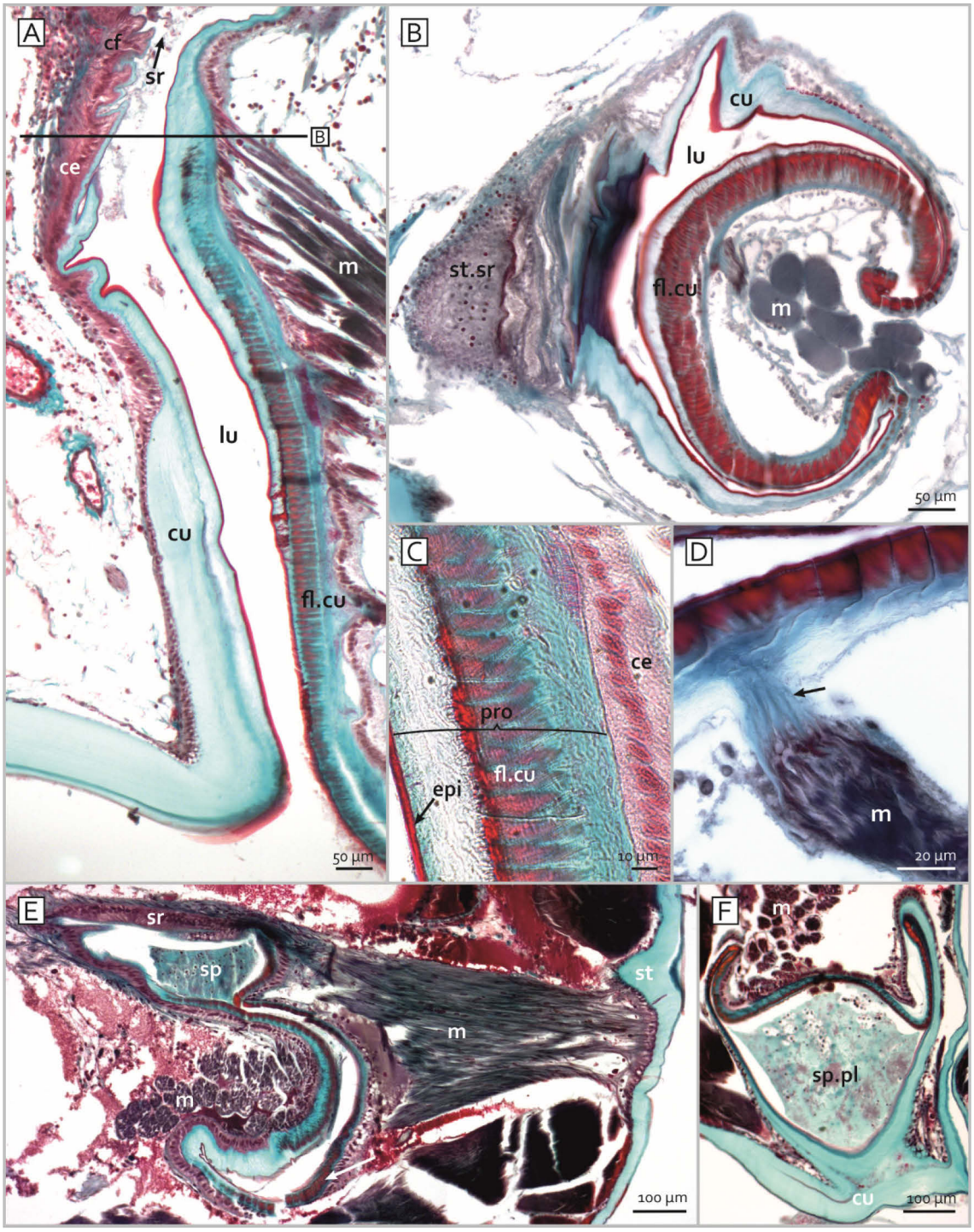

Fig. 2.7 Histological sections of vagina and associated structures of *Mithraculus sculptus* (A–D) and *Stenorhynchus seticornis* (E–F). **A**: Longitudinal section of vagina with attached muscles. The cuticle epithelium forms prominent folds at the transition to the seminal receptacle. The line indicates approximate position of cross section shown in B. **B**: Cross section of crescent shaped vagina at transition to seminal receptacle. The flexible inner wall is invaginated into the outer wall. **C**: Detail of the flexible vagina cuticle. Columnar epithelium lined by cuticle. Two cuticle areas can be distinguished: procuticle and epicuticle (the latter facing the lumen). **D**: Muscle attachment to the flexible cuticle of inner vagina wall. Arrow = fibrous tissue that connects the muscle to the cuticle.

2.4.2 | THE FEMALE REPRODUCTIVE SYSTEM

The morphology of the reproductive system in the investigated species follows the general pattern of heterotreme eubrachyurans including other Majoidea. The oviducts connect the paired ovaries to the likewise paired seminal receptacles, which in turn open to the vulvae through the vaginae. Furthermore, the ovaries, the oviducts, and the seminal receptacles are enclosed by connective tissue (Figs. 4D,F,G, 5D,E).

Whereas some studies focused only on some aspects of the majoid reproductive system such as the development of the ovaries (Hinsch & Cone, 1969; Rotllant et al., 2007), the whole reproductive system has been comprehensively described in the species *Chionoecetes opilio* (Beninger et al., 1988; Beninger et al., 1993; Lanteigne et al., 1996; Sainte-Marie & Sainte-Marie, 1998; Sainte-Marie et al., 2000; Benhalima & Moriyasu, 2001), *Hyas coarctatus* Leach, 1816 (Hartnoll, 1968; Lanteigne et al., 1996), *Hyas araneus* (Linnaeus, 1758) (Hartnoll, 1968), *Inachus phalangium* (Fabricius, 1775) (Diesel, 1989, 1991), *Stenorhynchus seticornis* (Antunes et al., 2016), *Maja brachydactyla* Balss, 1922 (Rotllant et al., 2007), *Libinia spinosa* (Sal Moyano et al., 2010; González-Pisani et al., 2011; Sal Moyano et al., 2011), *Leurocyclus tuberculosus* (H. Milne Edwards & Lucas, 1842) (González-Pisani et al., 2011). This broad knowledge offers the possibility to identify shared characters of the majoid reproductive system (for a summary see Table 2).

THE SHAPE OF THE OVARIES

The ovarian lobes of *Mithraculus sculptus* correspond to the organization of other Brachyura (McLay & Becker, 2015) and are consistent with the H-shape pattern, with the ovaries restricted to the cephalothorax (see Krol et al., 1992). The posterior fusion of the ovaries in *S. seticornis* (referred to as O-shape herein) is linked with an extension into the pleon. Interestingly, a similar extension of the ovaries has been described for species of three other majoid species (Rotllant et al., 2007; González-Pisani et al., 2011). Additionally, an extension of ovarian lobes into the pleon has previously been described for thoracotremes of the groups Grapsoidea (de Souza & Silva, 2009), Pinnotheridae (Becker et al., 2011) and Cryptochiridae (Vehof et al., 2016).

E: Cross section of crescent shaped vagina of S. *seticornis*. Muscles attached on both sides of vagina walls. Seminal receptacle filled with sperm mass. **F**: Cross section through vagina, with the sperm plug clearly visible in lumen.

Abbreviations: ce = columnar epithelium; cu = cuticle; epi = epicuticle; pro = procuticle; cf = cuticle folds; fl.cu = flexible part of cuticle; st.sr = secretory tissue of the seminal receptacle; m = muscle; sp.pl = sperm plug; sp = sperm mass; sr = seminal receptacle; st = sternum; lu = lumen (of vagina).

Although the macroscopic organization is quite similar, the cell arrangement of the developing oocytes in the ovaries differs from any known description. So far, the germinative zones of heterotreme ovaries were always described as situated centrally with oocytes wandering to the periphery during their maturation progress (Hinsch & Cone, 1969; Johnson, 1980; Rotllant et al., 2007). In all females investigated in the present study, this usual arrangement is expanded in a more complex manner with germinative zones and adjacent previtellogenic oocytes stretching through areas of mature oocytes (Fig. 4A–C). The very small ovaries of a freshly spawned female resemble the usual arrangement to some extent but this seems to be due to the stage of the reproductive cycle. This implies that changes, not only in general size, but also in the histology within the ovaries, depend on the female reproductive cycle. The absence of vitellogenic oocytes within the ovaries may indicate a seasonal reproduction or a rapid vitellogenesis.

THE OVIDUCT ORIGIN OR WHERE DO THE "FOLLICLE CELLS" FIT IN?

At first sight, "accessory-" or "follicle cells" seem to be distributed irregularly in between the developing oocytes (Fig. 4E). In some studies they have been interpreted as the cells that surround the developing oocytes (Hinsch & Cone, 1969) and form the chorionic membrane (Johnson, 1980; de Souza & Silva, 2009). Due to their distribution and arrangement within the ovary and oviduct it might be possible that in fact they are not randomly distributed, but the continuous epithelial cells of the convoluting oviduct and ovary strands (Fig. 4F).

Given that germinative zones and premature oocytes are also found within the oviduct in very close proximity to the seminal receptacle, the oviduct can be regarded as part of the ovary that forms the connection to the seminal receptacle (Fig. 4D,F). The structural similarity of oviduct and ovary and the view that they should not be treated as separate structures, have been previously discussed by several authors (Hard, 1942; Spalding, 1942; Hartnoll, 1968; Becker et al., 2011).

The oviduct does not form an open tube where it connects to the SR of *M. sculptus* and *S. seticornis*. Therefore, it seems likely that the tissues of the seminal receptacle and oviduct undergo cyclic changes and only form a tube when the female ovulates. This temporary orifice has been reported for the majoid *C. opilio* (Sainte-Marie & Sainte-Marie, 1998) and the grapsoid *Eriocheir sinensis* H. Milne Edwards, 1853 (Lee & Yamazaki, 1990).

THE SEMINAL RECEPTACLE AND THE ISSUE OF THE VELUM

In both investigated species the dorsal area of the SR is formed by a secretory tissue whose cells release secretions and degenerate towards the lumen (Fig. 5D). Secretory tissues have been described in numerous eubrachyuran species showing different dimensions within the SR (Johnson, 1980; Zara et al., 2014; Ewers-Saucedo et al., 2015; Hayer et al., 2015; de Souza et al., 2017).

Table 2.2 A comparison of the female reproductive system of investigated Majoidea. — **Abbreviations:** cgz = central germinative zone; H = H or X shape; O = posteriorly fused ovary lobes; sph = spermatophore; st = secretory tissue; cu = cuticle; + = yes; – = no; * = with muscle within the folds; ° = plus an additional muscle; *italics* = interpretation / disputable; **bold** = consistently in all investigated species; ? = no information available.

Group	Species	References	ovary		oviduct	seminal receptacle			vagina		
			morphology / dimension	cgz	orifice close to vagina	**structure at transition st – cu**	sperm distribution	sperm condition	**general / oblique muscle**	sperm plug	**vulva closure**
Oregoniidae	***Chionoecetes opilio***	Beninger et al., 1988, 1993; Lanteigne et al., 1996; Sainte-Marie & Sainte-Marie, 1998	H / carapace	+	+	**folds***	dorsal / ventral	sph	**concave/ +**	–	**inner vagina wall**
	Hyas araneus	Hartnoll 1968	H / carapace	?	+	**folds**	?	?	**concave/ +**	+	**inner vagina wall**
	Hyas coarctatus	Hartnoll 1968; Lanteigne et al., 1996	H / carapace	+	+	**folds***	?	?	**concave/ +**	?	**inner vagina wall**
Inachidae	***Inachus phalangium***	Diesel 1989, 1991	H / carapace	?	+ / *ventral chamber*	***velum***	dorsal	packages	**concave/ +**	+	**inner vagina wall**
Inachoididae	***Stenorhynchus seticornis***	this study; Antunes et al., 2016	O / pleon	–	+	**folds**	dorsal / ventral	free mass / sph	**concave/ +°**	+	**inner vagina wall**
	Leurocyclus tuberculosus	González-Pisani et al., 2011	O / pleon	?	*? intermediate*	**folds**	dorsal / ventral	free mass	**concave/ +**	–	**inner vagina wall**
Majidae	***Maja brachydactyla***	Rotllant et al., 2007	O / pleon	+	+	–	dorsal / ventral	free mass	**concave/ +**	?	?
	Mithraculus sculptus	this study	H / carapace	–	+	**folds**	dorsal / ventral	free mass	**concave/ +**	–	**inner vagina wall**
Epialtidae	***Libinia spinosa***	Sal Moyano et al., 2010, 2011; González-Pisani et al., 2011	O / pleon	?	+ / intermediate	***velum* / folds**	dorsal / ventral	packages	**concave/ +**	–	**inner vagina wall**

Interestingly, the arrangement of the secretory tissue cells at the proximity to the oviduct connection of the females of *M. sculptus* and *S. seticornis* follows a similar pattern as that described as the "holocrine transfer tissue" in Pinnotheridae described by Becker et al. (2011) (see also Antunes et al., 2016) (Fig. 6C,G). If this pattern is homologous, this would undoubtedly serve as a useful character but it needs further investigations into this subject to verify this.

Diesel (1989, 1991) described a division of the SR into two discrete chambers in females of a number of majoid species. The dorsal, secretory "storage chamber" and the ventral, cuticular "insemination chamber" were interpreted as a key aspect of majoid reproduction and discussed in terms of sperm competition (Diesel, 1989, 1991). According to this view, the velum that separates both chambers could allow the female to control the amount of sperm stored in the dorsal "storage chamber" and of that released into the ventral "insemination chamber" during ovulation. The concept of the velum has been adopted by some authors for other majoids and a number of eubrachyuran species (e.g., *L. spinosa*: Sal Moyano et al. (2010); González-Pisani et al. (2011); *Ucides cordatus* (Linnaeus, 1763): Sant'Anna et al. (2007)). In the present study however, none of the females possesses a velum, which challenges previous observations. In some sections of *S. seticornis* a structure similar to a velum appears but the three dimensional reconstruction reveals it to be an invagination of a cuticle-lined area of the seminal receptacle wall (see * in Fig. 6D,F), which does not separate it into two chambers. In *M. sculptus* a structure resembling the velum in *L. spinosa* (González-Pisani et al., 2011) is present, but is just a prominent cuticle bulge that protrudes into the ventral area-lumen of the SR and stretches towards the opposite wall (Fig. 5A4).

Thus, it might be necessary to differentiate between a velum in the sense of Diesel (1989) and cuticle invaginations that incompletely divide the ventral area of the seminal receptacle.

Instead of a velum, in most of the investigated majoid species some cuticle folds are present at the transition between the dorsal and ventral area of the seminal receptacle (see Table 2 for a summary of the hitherto investigated majoid species). These folds can be structurally different. In *C. opilio* and *H. coarctatus* musculature inserts into the cuticle folds (Beninger et al., 1993; Lanteigne et al., 1996), whereas in all other species muscles are absent. Antunes et al. (2016) detected musculature within the folds in *S. seticornis*. However, this finding has not been confirmed in our study. The presence of cuticle folds seems to be a widely distributed eubrachyuran character (Becker et al., 2011; González-Pisani et al., 2011; de Souza et al., 2013). Nevertheless, with the sperm mass being present in the entire lumen of the seminal receptacle, the cuticle folds seem not to limit its dispersion. Thus, a division in a sperm "storage- and insemination chamber" as described by Diesel (1989) is unlikely. Due to the lack of a velum or other structures such as a bursa (Vehof et al., 2017), an active participation of the female during copulation regarding the amount of sperm used for fertilisation and control over specific male sperm seems improbable.

Antunes et al. (2016) observed spermatophores and free spermatozoa in the ventral region of the SR of *S. seticornis*. In contrast to this, we observed only free spermatozoa in *M. sculptus* and *S. seticornis*, which might be due to differences in the elapsed time since mating. The absence of spermatophores and sperm layering in the seminal receptacle of both species could also be due to this. Similar conclusions have been drawn for hymenosomatids as sperm masses from multiple copulations slowly mix after some time (van den Brink & McLay, 2009; Klaus et al., 2014).

Concerning the oviduct orifice of eubrachyurans, Diesel (1991) differentiated between a SR of a *dorsal-type* and a *ventral-type*, with the oviduct orifice being located opposite to or adjoining the vagina, respectively. This differentiation has been widely accepted and further hypotheses concerning sperm competition were built upon it (Diesel, 1991; McLay & López-Greco, 2011). In the herein studied species, the oviduct connection with the secretory tissue of the seminal receptacle is situated somewhat intermediate. Nevertheless, it is situated close to the cuticle area and the vagina and therefore of the ventral type (Figs. 5, 6). With regard to sperm competition, this would indicate a last male precedence. Yet, since no layering is obvious, it remains unclear if the stored sperm belongs to more than one male.

THE VAGINA

The continuity of the cuticle in the seminal receptacle, the vagina, and the integument suggests an ectodermal origin of all these structures. The structures of the vagina have been elaborated in detail by Hartnoll (1968). In his study on brachyuran female genital ducts he recognized four types of vaginas, namely (1) simple, (2) concave and concave with operculum (3) mobile, and (4) immobile.

All hitherto investigated majoid species have vaginae of the concave type and the vulva is enclosed by the deflated inner wall and opens only by contraction of the attached muscle. Hartnoll (1968) refers to this type of closure of the genital ducts as operculum.

2.5 | CONCLUSIONS

Several characters are shared by the species investigated in the present study (see also tables 1 and 2):

Male gonopods. (1) The G1 is long, slender, tapers distally and forms a bulbous tip. **(2)** The opening of the ejaculatory canal is subterminal and **(3)** surrounded by denticles. **(4)** The gonopod tegumental glands (= rosette glands) are present in the proximal part of the G1 where the G2 is inserted. **(5)** The G2 is short and stout and **(6)** has longitudinal folds on its distal surface and **(7)** an apical girdle is present around its distal tip. **(8)** The penis emerges from the gonopore of the fifth coxa and enters the G1 opposite the G2.

Female reproductive system. Within the SR **(1)** a (mostly) dorsal secretory area can be distinguished from **(2)** a (mostly) ventral area lined by cuticle. **(3)** Both areas are separated by cuticle folds with or without muscle attachment. **(4)** The vagina is always of the concave pattern (sensu Hartnoll 1968) and **(5)** the vulva is enclosed by the inner flexible wall of the deflated vaginal tube.
In contrast to earlier descriptions of majoid reproductive systems, the species investigated in the present study lack a division of the seminal receptacle into a dorsal sperm "storage chamber" and a ventral "insemination chamber" separated by a muscular velum. Instead, we observed invaginations of the cuticle receptacle wall in histological sections and 3D-reconstructions which represent no anatomical or functional division of the seminal receptacle. In histological sections however, those invaginations resemble the data published by Diesel (1989, 1991) and could by mistake be interpreted as a velum. At the present stage, it remains unclear whether a divided seminal receptacle is a character which is only present in part of the Majoidea or whether histological observations of earlier studies have been misinterpreted. Our findings clearly show the benefit of 3D-reconstruction to understand the spatial organisation of reproductive structures and suggest a re-consideration of the velum as a majoid character.

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REFERENCES

For all citations provided, please see the concatenated reference list at the end of this thesis

3 | THE MORPHOLOGY OF THE REPRODUCTIVE SYSTEM IN THE CRAB *PERCNON GIBBESI* (DECAPODA: BRACHYURA: GRAPSOIDEA) REVEALS A NEW COMBINATION OF CHARACTERS IN THORACOTREMATA

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ABSTRACT

Recent studies have revealed a high diversity of reproductive structures in heterotreme brachyurans, while those of Thoracotremata seem rather uniform. Yet, there still is a huge lack of data in this group as only few species have been studied with respect to their reproductive system. The phylogenetic position of Percnidae is ambiguous. Recent molecular studies place it within polyphyletic grapsoids. We herein study the reproductive morphology of *Percnon gibbesi* using histology, scanning electron microscopy, micro-computed tomography and 3D-reconstructions to test whether this species shows the characteristic thoracotreme pattern. Our results reveal that the male copulatory system conforms to other thoracotremes. It is composed of two pairs of pleopods (gonopods) and likewise paired penes. The first gonopod is relatively long. It possesses a bent terminal process with a distal opening of the ejaculatory canal, a character also present in other grapsoids. The second gonopod is short and terminates in an apical girdle. The female reproductive system reveals a combination of characters, so far unknown for thoracotremes. The paired oviducts do not lead into the seminal receptacles, but run into separate cuticular ducts joined with the vaginae. Accessory sperm storage organs, the bursae, are also connected to the vaginae. Bursae have previously only been described in heterotreme crabs. The data presented in this study reveals a higher diversity of thoracotreme reproductive systems than anticipated.

KEYWORDS: bursa, fertilisation, gonopods, holocrine transfer tissue, separate oviduct orifice, terminal process

3.1 | INTRODUCTION

The eubrachyuran crab *Percnon gibbesi* (H. Milne Edwards, 1853) gained recent attention as an invasive decapod species entering the Mediterranean (Yokes & Galil, 2006). The natural distribution of this species is in temperate to tropical waters of the East-Pacific and Atlantic from where it has entered the Mediterranean through the Strait of Gibraltar (Yokes & Galil, 2006). Being first recorded on the coasts of several western and central Mediterranean islands from 1999 onwards (Relini, Orsi, Puccio & Azzurro, 2000; Mueller, 2001; Deudero, Frau, Cerda & Hampel, 2005), it has spread eastwards, reaching the most eastern Mediterranean up to the coasts of Israel in less than a decade (Ilan, Shlagman, Goren, Shema & Galil, 2015). The phylogenetic relationships of Grapsoidea and the position of the genus *Percnon* have been subject to a controversial debate and several changes (see Schubart et al. 2000, 2006; Števčić 2005). Based on adult morphological characters, *Percnon* was originally placed in the Plagusiidae. However, in a more recent molecular study, Percninae have been excluded from the Plagusiidae and elevated to Percnidae (Schubart & Cuesta, 2010). In this analysis, the Percnidae were resolved as the sister group to the remaining grapsoids including ocypodids. Likewise the molecular analysis of Tsang et al. (2014) did not show Grapsoidea as monophyletic.

The traditional subdivision of Eubrachyura into heterotreme and thoracotreme crabs had been based on the location of gonopores, i.e. the male and female sexual openings (Guinot, 1977). In heterotremes, males have coxal and females sternal gonopores, in thoracotremes the gonopores of both sexes are sternal (Guinot, 1977; Guinot, Tavares & Castro, 2013; Davie, Guinot & Ng, 2015). The male copulatory systems of eubrachyurans show a great morphological and functional diversity. As is true for Brachyura in general, the anteriormost two pairs of pleonal appendages, the first and second gonopods, are used for sperm transfer. During copulation the second gonopod (G2) is generally inserted into to the tubular first gonopod (G1). In some heterotremes the G2 can be longer than the G1, thereby protrude from its distal opening when inserted, and function directly in the transfer of sperm (Brandis, Storch & Türkay, 1999; Klaus, Schubart & Brandis, 2006; Ewers-Saucedo, Hayer & Brandis, 2015). In other heterotremes and in all Thoracotremata the G2 is clearly shorter than the G1 and has a rather accessory role while the G1 transfers the sperm into the female ducts (Lautenschlager, Brandis & Storch, 2010; Becker, Türkay & Brandis, 2012; Guinot et al., 2013; McLay & Becker, 2015). Corresponding to the male reproductive systems, those of the females show a high degree of variation in heterotremes. In contrast to this they are rather uniform in the species of Thoracotremata studied so far (McLay & Becker, 2015). However, heterotremes and thoracotremes have been generally considered as exhibiting internal initiation of fertilisation as each of the paired oviducts is directly connected to the paired internalised sperm storage organs, the seminal receptacles (but see Vehof et al. 2018).

Traditionally, the Thoracotremata comprises four groups, the Ocypodoidea, the Grapsoidea, the Cryptochiroidea, and the Pinnotheroidea (Ng, Guinot & Davie, 2008; Davie et al., 2015). Yet the internal and external relationships of these groups are still controversial (Tsang et al., 2014). Nevertheless, the reproductive systems of representatives of all four groups have been investigated (Varunidae: Lee & Yamazaki, 1990; Ocypodidae: López-Greco, Fransozo, Negreiros-Fransozo & Dos Santos, 2009; Lautenschlager et al., 2010; Pinnotheridae: Becker et al., 2011; Becker et al., 2012; Gecarcinidae: de Souza et al., 2013; de Souza et al., 2017; Cryptochiridae: Vehof et al., 2016). However, considering the diversity of thoracotremes, there is still a huge lack of data concerning the reproductive systems of this group.

We herein present a detailed study on the male copulatory and female reproductive system of *P. gibbesi* using histology, scanning electron microscopy, micro-computed tomography and 3D-reconstructions. The goal of the present study is to test whether *P. gibbesi* conforms to the characteristic pattern in the reproductive morphology of thoracotremes.

3.2 | MATERIAL AND METHODS

3.2.1 | MATERIAL

Four female and three male specimens of *Percnon gibbesi* (H. Milne Edwards, 1853) were obtained from commercial vendors (www.shop-meeresaquaristik.de).

3.2.2 | HISTOLOGY

For the histological analyses, all four female specimens were cold-anaesthetised in a freezer at –18°C for 15 minutes. Whole specimens were preserved either in Bouin's solution or in "Susa Heidenhain" (MORPHISTO® Evolutionsforschung und Anwendung GmbH, Frankfurt am Main, Germany) for 48 hours. For decalcification, specimens were treated in Ethylenediaminetetraacetic acid (EDTA) for 48–72 hours. Specimens were then dehydrated through an ascending series of ethanol solutions and infiltrated (Shandon Hypercenter XP, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and embedded with paraffin. Sections were prepared at 6–8 µm using a Leica RM2255 rotary microtome (Leica Microsystems GmbH, Wetzlar, Germany). All histological sections were stained with the trichromatic Masson-Goldner "light green" protocol (MORPHISTO®, Frankfurt am Main, Germany).

3.2.3 | SCANNING ELECTRON MICROSCOPY (SEM)

The dissected gonopods were cleaned manually after an ultrasonic bath. The first and second gonopods were critical point dried (Bal-Tec CPD 030, Balzers, Liechtenstein) and sputter coated with

a gold layer (Bal-Tec SCD 005, Balzers, Liechtenstein). The micrographs were taken using a LEO 1430 scanning electron microscope (Carl Zeiss Nano Technology Systems GmbH, Oberkochen, Germany) and images were processed with Corel Draw X6 software (Corel, Ottawa).

3.2.4 | MICRO-COMPUTED TOMOGRAPHY (μCT)

One male specimen, with the second gonopod still inserted into the first one, was fixed in "Susa after Heidenhain" (MORPHISTO®, Frankfurt am Main, Germany) for 48 hours and washed repeatedly in 70% ethanol. After dissecting the pleon together with the attached gonopods, the sample was dehydrated through a series of ethanol solutions. For contrast improvement, the sample was immersed in a 1% iodine-ethanol solution for 24 h and subsequently critical point dried (Bal-Tec CPD 030, Balzers, Liechtenstein).

The μCT scan was conducted using a Phoenix nanotom X-ray|s tube at 100 kV and 150 μA, generating 2000 projections. The effective voxel size was 20 μm, the detector timing 750 ms. The cone beam reconstruction was performed using datos|x-reconstruction (GE Sensing and Inspection Technologies GMBH Phoenix|x-ray) and a stack of virtual sections was produced and exported with VGStudio Max software (Volume Graphics, Heidelberg).

3.2.5 | 3D-RECONSTRUCTION, PHOTOGRAPHY AND IMAGE PROCESSING

The reconstruction of 3D-models based on histological sections and μCT scans was carried out with Amira software (FEI Visualization Sciences Group, Bordeaux). Histological serial sections were viewed and photographed using an Axioskop 2 stereo microscope equipped with an Axio Cam HRc camera using AxioVision 4.3 software (Carl Zeiss Vision GmbH). The images were converted into grey scale and aligned. Based on different gray scale values, the contours of each reproductive structure were labeled throughout the digital image stack and then used to calculate a surface model of the reproductive system. The 3D-reconstruction of the μCT scans was carried out by processing image stacks of virtual sections. These sections were then edited the same way as the aligned histological sections.

In situ multi-scan images of the male copulatory system were taken with a Keyence VHX1000 digital microscope (Keyence, Ōsaka, Japan). All images were processed and assembled in figure plates using Corel Draw X6 and Corel Photopaint X6 software (Corel, Ottawa).

3.3 | RESULTS

3.3.1 | MALE

The male copulatory system of *Percnon gibbesi* consists of the paired penes and paired first and second gonopods (G1 and G2) located symmetrically on each body half (Figs. 3.1-3.3). The penis is a small, translucent tube and the external extension of the ejaculatory duct. It emerges from the gonopore on sternite 8 (Fig. 3.1A,B), and is accompanied by an elongation of episternite 7. The gonopore is situated close to the coxa of pereiopod 5 (Fig. 3.1C).

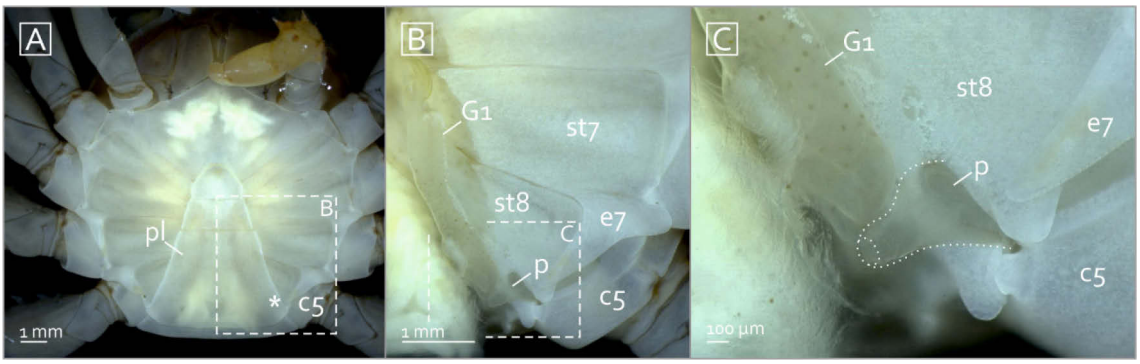

Fig. 3.1 *Percnon gibbesi*, male copulatory system (Keyence digital microscope). **A**: Ventral view of the male pleon. * indicates the position of the gonopore **B**: Close-up of the left side with first gonopod and penis visible. (C): The gonopore on sternite 8 and penis.
Abbreviations: c5 – coxa of pereiopod 5, e7 – episternite 7, G1 – first gonopod, p – penis, pl – pleon, st7/8 – sternite 7/8

The G1 comprises a proximal and a distal podomere (Fig. 3.2A). The proximal podomere is short and stout and articulates the G1 to the pleon. The distal podomere is long, dorsoventrally flattened and has a terminal process (Figs. 3.2Aa; 3.3A-C). It forms a tube, the ejaculatory canal, with a wide proximal and a narrow distal opening (Figs. 3.2A,C; 3.3B,C). The course of the ejaculatory canal is indicated by a suture that runs diagonally along the dorsolateral surface of the G1 (Fig. 3.3A). The opening of the ejaculatory canal is situated most distally on a terminal process and directed towards anteromedial. Gonopod tegumental glands (rosette glands) are situated inside the base of the distal podomere of the G1, in proximity to the proximal opening of the ejaculatory canal (Fig. 3.2B,C). Two muscles connect the proximal and distal podomere of the G1 (Fig. 3.2A,C,D). One muscle (m1a) has its origin within the dorsolateral proximal podomere. A second muscle (m1b) originates from two areas dorsolateral and ventromedial inside the proximal podomere. Both muscles have separate projections towards the area where the proximal and distal podomeres meet (Fig. 3.2A,C,D). Two different types of setae are present on the G1 (Fig. 3.3A-D). Pappose setae are distributed around the proximal opening of the distal podomere (Fig. 3.3A,D) and simple setae are

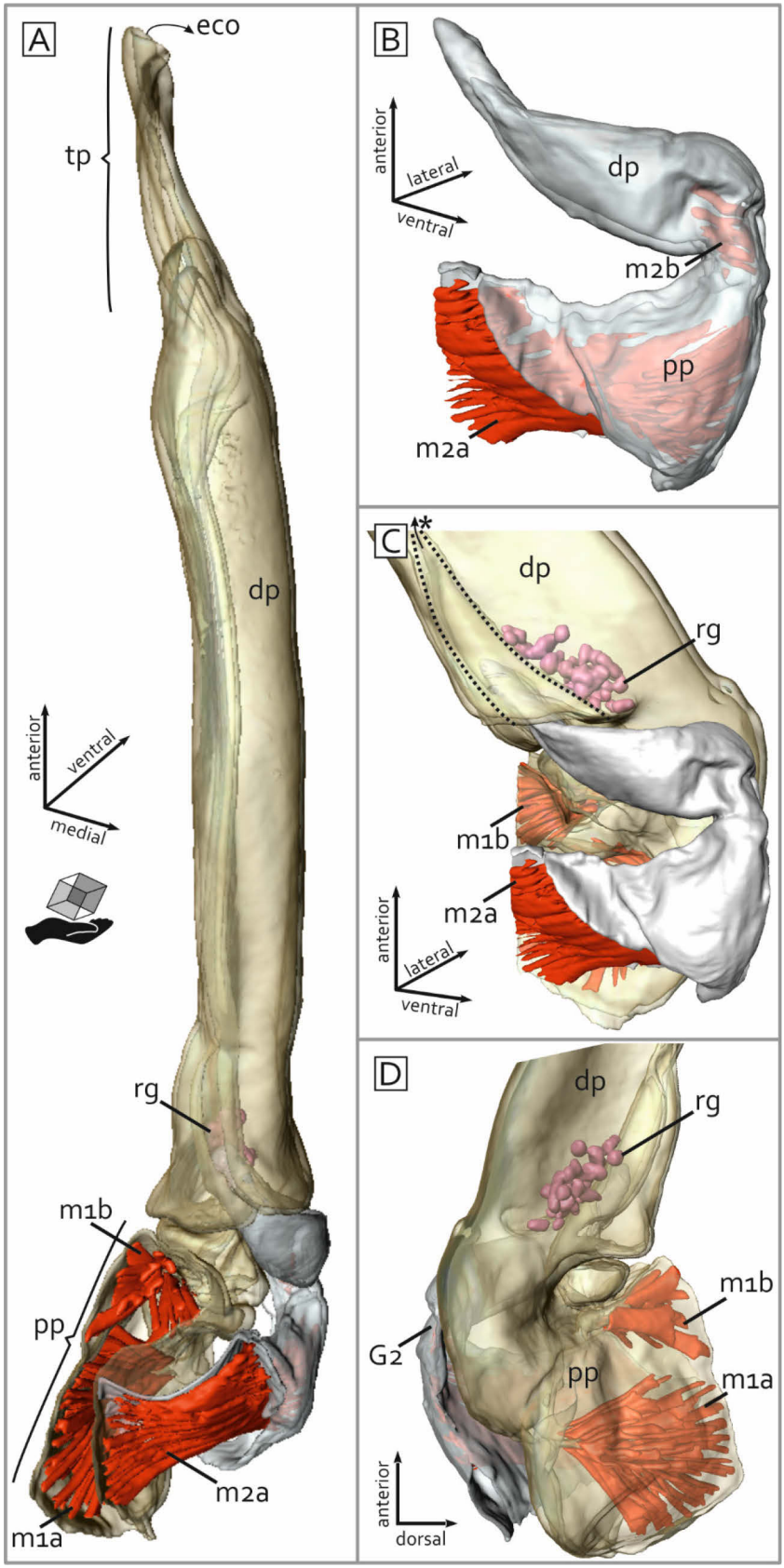

Fig. 3.2 Male *Percnon gibbesi*, three dimensional-reconstruction of the first and second gonopods (left side) **A** The first gonopod (beige colour-ation) with the basally inserted second gonopod (grey colouration). The surfaces of the podomeres are semi-transparent. View from dorso-medial. **B**: The second gonopod. View from ventro-medial. **C**: The second gonopod is inserted into the first gonopod (semi-transparent surface). The pleopod tegumental glands (rosette glands) are situated in proximity of the basal opening of the ejaculatory canal. The ejaculatory canal (indicated by dashed lines) narrows distally (arrow with *). View from ventro-medial. **D**: The basal part of the first gonopod and the muscle strands in the proximal podomere. (* indicates the insertion site of the penis). Lateral view.
Abbreviations:
dp – distal podomere,
eco – ejaculatory canal opening,
G1 – first gonopod,
G2 – second gonopod,
m1a – muscle 1 of G1,
m1b – muscle 2 of G1,
m2a – muscle 1 of G2,
m2b – muscle 2 of G2,
rg – rosette glands,
pp – proximal podomere,
tp – terminal process.
Panel **A** is an interactive 3D-model available in the digital version.

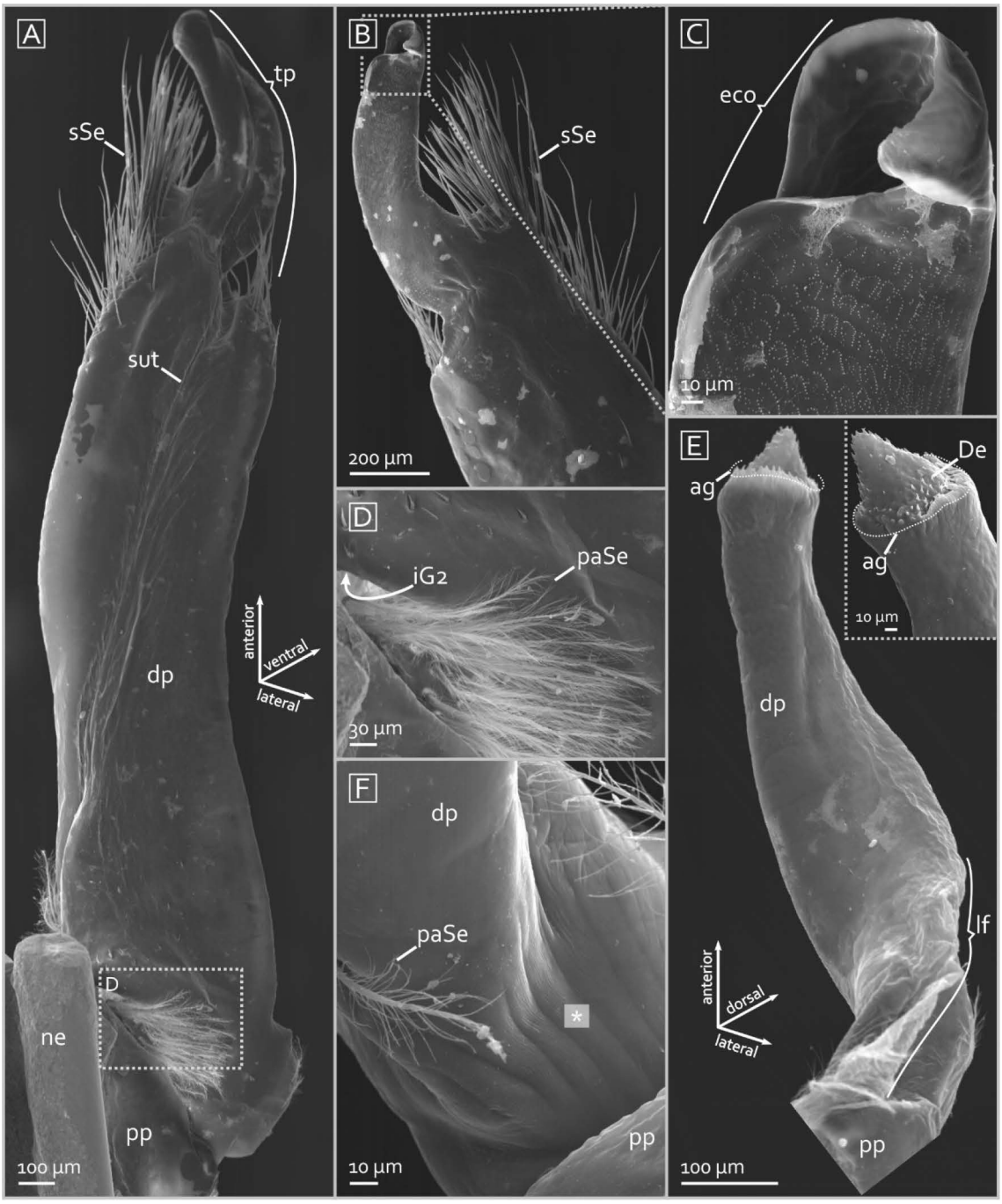

Fig. 3.3 *Percnon gibbesi*, scanning electron micrographs of the first and second gonopods (right side). **A**: The first gonopod; the suture indicates the course of the ejaculatory canal inside the distal podomere. **B**: The ejaculatory canal opens distally on the bent terminal process. Simple setae arise at its base. **C**: The ejaculatory canal opening; note the distinctive pattern of fine cuticle surface structures on the terminal process. **D**: Detail of pappose setae on the first gonopod, grouped around the insertion area of the second gonopod. **E**: The second gonopod; the distal podomere bears an apical girdle with denticles around its tip (see detail). **F**: Longitudinal folds (*) along the medial surface between the proximal and distal podomere of the second gonopod.

Abbreviations: ag – apical girdle, De – denticle, dp – distal podomere, eco – ejaculatory canal opening, iG2 – insertion area of second gonopod, lf – longitudinal fold, ne – dissecting needle, paSe – pappose setae, pp – proximal podomere, sSe – simple setae, sut – suture, tp – terminal process

present at the base of the terminal process (Fig. 3.3B). Additionally, many very small cuticle spines form a distinct pattern on the terminal process (Fig. 3.3C). The stout G2 is formed by a proximal and distal podomere and equipped with two prominent muscles (Fig. 3.2B). One muscle (m2a) connects the proximal podomere to the pleon (Fig. 3.2A-C). The second muscle (m2b) has both its origin and projection within the base of the distal podomere, running from ventral to dorsal (Fig. 3.2B). The cuticle of the G2 is predominantly smooth but has a prominent longitudinal fold on its lateral edge at the transition of the proximal and distal podomere (Fig. 3.3E). Medially, on the opposite side, several small longitudinal folds are present (Fig. 3.3F). The tip of the distal podomere forms an apical girdle (sensu Beninger et al., 1991). Additionally, a few pappose setae with a patchy distribution are present at the proximal podomere and the basal part of the distal podomere (Fig. 3.3E,F).
The penis and the G2 are inserted into the base of the proximal podomere of the G1 during copulation. They enter the G1 at different positions on opposite sides, but share the same lumen within the most proximal part of the ejaculatory canal.

3.3.2 | FEMALE

The female reproductive system of *P. gibbesi* consists of several paired organs. Sperm is stored in seminal receptacles and accessory sperm storage structures, the bursae. The oviduct does not enter the seminal receptacle directly but leads into a cuticular duct between seminal receptacle and bursa (Fig. 3.4).

OVARY AND OVIDUCT

The ovaries are restricted to the thorax in *P. gibbesi* and show a characteristic organisation. The germinative zones occupy a central position in the ovary lobes, where oogonia proliferate and develop into oocytes. These mature and undergo vitellogenesis during their transport to the peripheral maturation zones. The most mature (vitellogenic) oocytes are situated along the outer margins of the ovary. In all specimens investigated vitellogenic oocytes were present in the ovary (Fig. 3.5A).
The oviduct arises as part of the ovary and runs medially alongside the whole length of the seminal receptacle towards the vagina. It leads into a cuticle-lined duct connected to the vagina. The oviduct orifice is therefore separate from the seminal receptacle lumen (Figs. 3.4; 3.5B,C,E,F) but close to the junction between seminal receptacle, vagina, and bursa (Fig. 3.5B,C). The cuticle epithelium of the duct surrounding the oviduct orifice is corrugated. The oviduct leads into a holocrine transfer tissue (sensu Becker et al., 2011) which consists of densely packed cells with oval nuclei and secretes a homogenous, orange-staining substance into the cuticular duct (Fig. 5F).

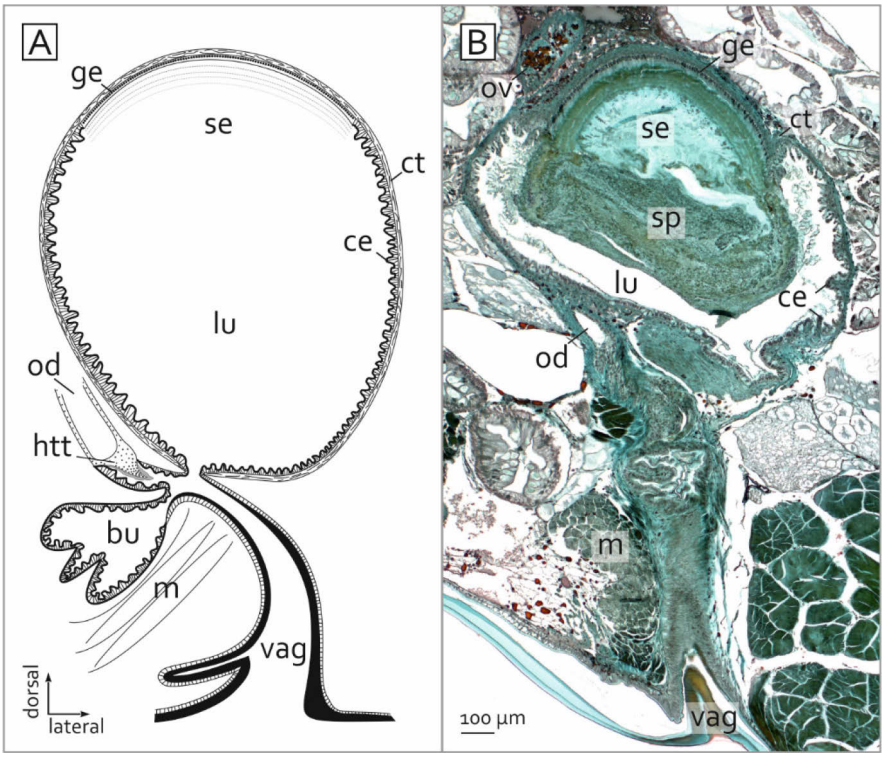

Fig. 3.4 *Percnon gibbesi*, overview of the female reproductive system in a schematic drawing **A** and a histological section **B**. The oviduct orifice inside the cuticular duct and the bursa are not shown in this section (female in late premoult stage).
Abbreviations: bu – bursa, ce – cuticle epithelium, ct – connective tissue, ge – glandular epithelium, htt – holocrine transfer tissue, lu – lumen, m – muscle, od – oviduct, ov – ovary, se – secretion, sp – sperm mass, vag – vagina

SPERM STORAGE AND VAGINA

The seminal receptacle is spherical and enclosed by connective tissue and a considerable amount of muscle fibres. It is predominantly lined by a corrugated cuticle epithelium (Fig. 3.6C,D). The most dorsal part of the seminal receptacle is lined by a glandular mono-layered columnar epithelium consisting of regularly shaped cells with large, basally located nuclei. The substances released by this epithelium are secreted in layers into the seminal receptacle lumen (Fig. 3.6A,B).
In all specimens studied, the lumen of the seminal receptacle was filled with free spermatozoa and different kinds of secretions but distinct sperm layering or male substances (sperm gel) were not observed (Fig. 3.6B-D).
An additional, smaller sperm storage structure, the bursa, is present in close proximity to the cuticular duct with the oviduct orifice and connected to the dorsal part of the vagina (Fig. 3.5B-D). The bursa is enclosed by connective tissue and muscles, and internally lined by a corrugated cuticle epithelium similar to the one observed in the cuticular duct and seminal receptacle.

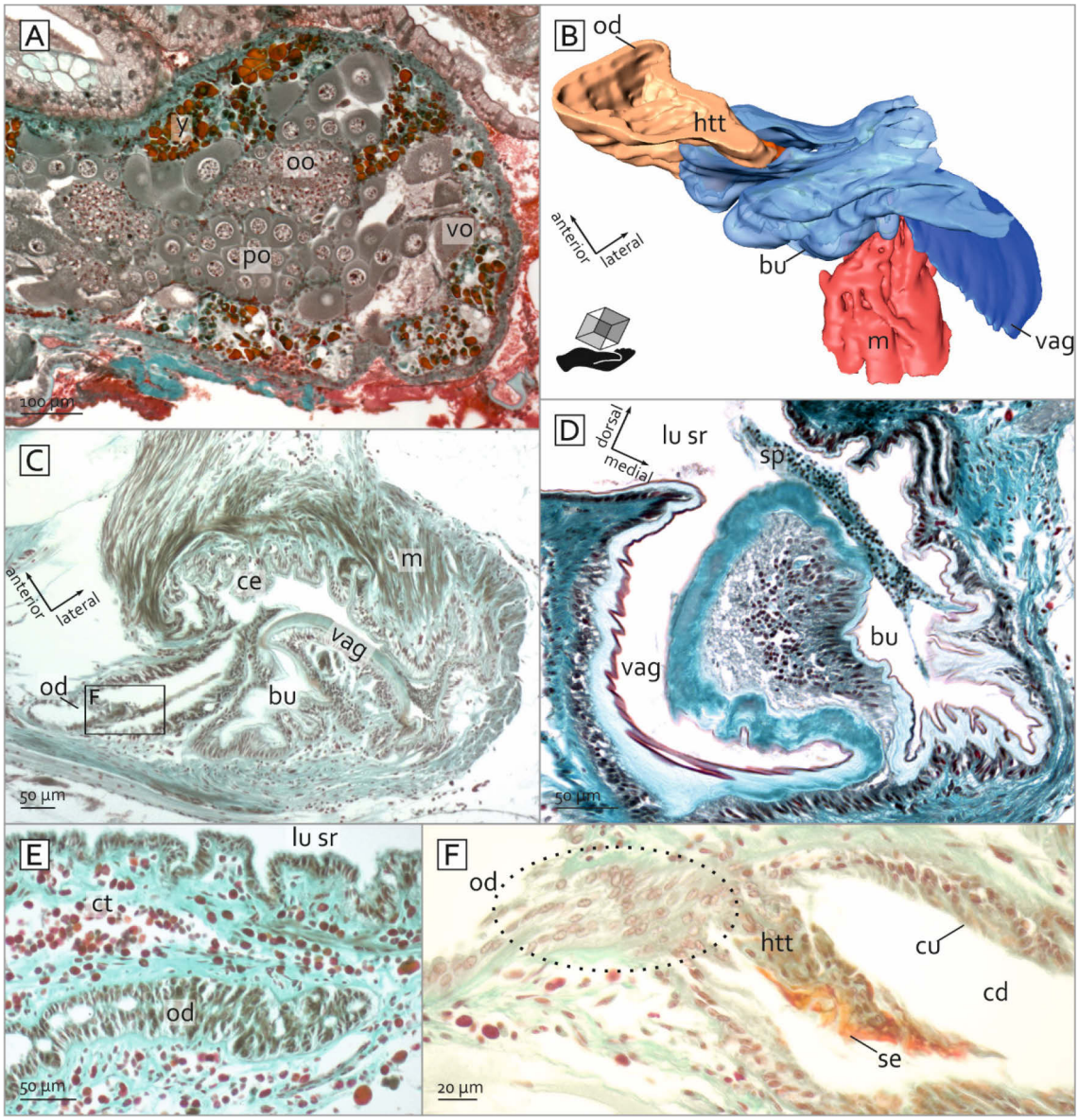

Fig. 3.5 Histological sections (**A**, **C-F**) and 3D-reconstruction (**B**) of ovaries, oviduct and bursa of female *Percnon gibbesi*. **A**: The cell arrangement within the ovary; centrally positioned oogonia and previtellogenic oocytes and laterally arranged vitellogenic oocytes. **B** The oviduct orifice leads into a secretory tissue (holocrine transfer tissue) that is enclosed into a cuticular duct separate from the seminal receptacle (not shown). **C**: Cross section through the oviduct orifice, bursa and inner vagina wall. View from dorsal. **D**: Sperm-filled bursa at the junction with the vagina and seminal receptacle. View from anterior. **E**: The oviduct orifice is situated ventrally of the seminal receptacle. **F**: The oviduct orifice (circle in dashed line) and the holocrine transfer tissue with (orange staining) secretions within the cuticular duct.
Abbreviations: bu – bursa, cd – cuticular duct, ce – cuticle epithelium, ct – connective tissue, cu – cuticle, htt – holocrine transfer tissue, lu sr – lumen of seminal receptacle, m – muscle, od – oviduct, oo – oogonia, po – previtellogenic oocyte, se – secretion, vag – vagina, vo ─ vitellogenic oocyte, y – yolk. Panel **B** is an interactive 3D-model available in the digital version.

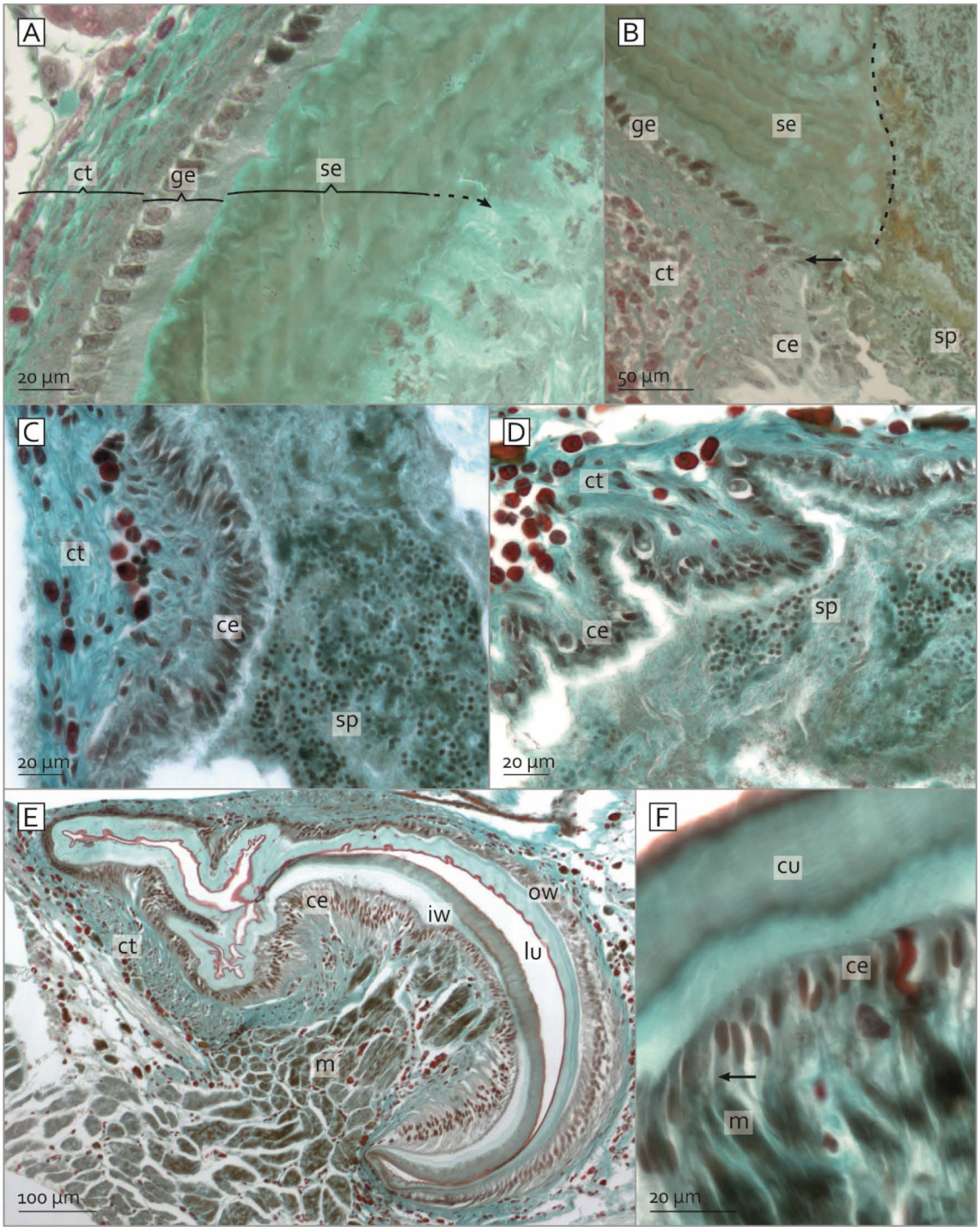

Fig. 3.6 Histological sections of the seminal receptacle and the vagina of female *Percnon gibbesi*. **A**: The monolayered glandular epithelium lines the seminal receptacle dorsally. The secretions appear layered. **B**: The transition of glandular and cuticle epithelium in the seminal receptacle (arrow). **C**: The cuticle epithelium that forms the main part of the seminal receptacle. Sperm and secretions are mixed and show no specific layering. **D**: The cuticle epithelium is corrugated and surrounded by connective tissue. **E**: Cross section of the crescent shaped vagina. The muscular inner vagina wall is invaginated into the outer wall. **F**: Detail of the inner vagina wall showing the insertion of muscle fibres in the cuticle epithelium (arrow).
Abbreviations: ce – cuticle epithelium, ct – connective tissue, cu – cuticle, ge – glandular epithelium, iw – inner vagina wall, lu - lumen, m - muscle, ow – outer vagina wall, se – secretions, sp - sperm

The bursae of one specimen were filled with sperm (Fig. 3.5D), while other specimens had empty, slightly crumpled bursae (Fig. 3.5B,C).
The vagina is of the concave type (sensu Hartnoll, 1968) and runs diagonally and slightly curved from the seminal receptacle posteriorly to the vulva in sternite 6. Musculature inserts along the flexible inner vagina wall, which is invaginated into the outer vagina wall, giving the vagina lumen a crescent shape in cross sections (Fig. 3.6E,F). The cuticle of the inner vagina wall stains slightly differently from the outer vagina wall. Sperm plugs were not observed in the vaginae.

3.4 | DISCUSSION

3.4.1 | THE MALE COPULATORY SYSTEM

The male copulatory system of *Percnon gibbesi* conforms to that of other thoracotreme eubrachyurans, with the penis emerging from the gonopore in sternite 8 (Guinot et al., 2013) and a long first gonopod (G1) that transfers the sperm into the female ducts (e.g. Pinnotheridae: Becker et al., 2012; Majoidea: Kienbaum et al., 2017). The second gonopod (G2) is short and has presumably an accessory role in the sperm transfer. The penis of Brachyura is not the intromittent organ but represents the outermost extension of the ejaculatory duct of the reproductive system of males (Minagawa et al., 1994; Garcia & Silva, 2006; Castilho et al., 2008; Becker et al., 2012; Guinot et al., 2013). It has been described as being equipped with muscles in several brachyuran species (Minagawa et al., 1994; Castilho et al., 2008; Zara et al., 2012). In *P. gibbesi* the penis emerges through the gonopore on sternite 8. Due to its close proximity to peraeopod 5, its position appears coxal as in heterotreme brachyurans; however, Guinot et al. (2013) demonstrated through dissections that the gonopore is indeed sternal.

THE FIRST GONOPOD (G1)

The most striking feature of the G1 is the terminal process with the distal opening of the ejaculatory canal. Similar processes have been described in several representatives of Grapsoidea and seem to be a common character in this group (Schubart et al., 2002; Naderloo & Schubart, 2010; Naderloo, 2011). Accessory distal structures of the G1 are also present in representatives of heterotremes such as Panopeidae (Martin & Abele, 1986), Palicidae (Castro, 2000) or Epialtidae (Sal Moyano et al., 2011). However, the position of the ejaculatory canal opening in the above mentioned heterotremes is sub-terminal while it is distal-most in the G1 in *P. gibbesi* and other representatives of the grapsoids.
One possible interpretation for such terminal processes is a "lock-and-key" principle and a role in reproductive isolation (Masly, 2011). The specialized processes in *P. gibbesi* and other species of

Grapsoidea may be closely coapted to corresponding structures in the female vulvae and/or vaginae and thereby prevent interspecific mating attempts. Terminal processes may also play a role in the precise placement of sperm. Due to the morphology of the vagina and associated cuticle structures in *P. gibbesi*, it seems likely that only the terminal process of the G1 becomes inserted into the vagina during copulation. The clusters of simple setae around the basis of the process may play a direct role in copulation, e.g. as mechanoreceptors that guide the G1 into position, as setae are well known to be part of the sensory system in crustaceans (Derby, 1989; Crouau, 1997; Garm & Høeg, 2006).
Small cuticle projections, very short setae or denticles along the tip of the G1 have also been described in several grapsoid species (Naderloo & Schubart, 2010; Naderloo, 2011) and in heterotremes, e.g. Bythograeidae: Tsuchida & Fujikura (2000), Cancridae: Moriyasu et al. (2002), Epialtidae: Sal Moyano et al. (2011), Calappidae: Ewers-Saucedo et al. (2015); Ewers-Saucedo et al. (2016). The pappose setae that are grouped around the proximal opening in the G1 have previously been reported from gonopods of other species (Beninger et al., 1991; Minagawa, 1993; Brandis et al., 1999; Kienbaum et al., 2017). Due to their fine structure and position in *P. gibbesi* these setae may act as filter structures, preventing particles to enter the ejaculatory canal.

SPERM TRANSPORT AND TRANSFER MECHANISM

The mechanisms of sperm transport by male gonopods and the transfer into the female ducts is variable among Brachyura and poorly understood since functional analysis from direct observations on sperm transfer are not available. The relative length of the gonopods constrains their role in sperm transfer (McLay & Becker, 2015). For example, the G2 transmits the sperm if it is longer than the G1, which is the case in several groups of heterotreme eubrachyurans (Orensanz et al., 1995; Brandis et al., 1999; Klaus et al., 2006; Ewers-Saucedo et al., 2015). In other heterotremes (Spalding, 1942; Johnson, 1980; Diesel, 1989) and all thoracotremes (McLay & Becker, 2015), including *P. gibbesi*, the tubular G1 is the transmitter of sperm and always longer than the G2. During copulation the penis is inserted into the G1 through a basal opening on the opposite side of the opening for the short G2. Both lumina are connected through a narrow passage within the ejaculatory canal a little more distally. Inserted into the G1, the G2 is supposed to have an accessory role in the transport of sperm and as a seal of the proximal opening in the G1 to the outside (Becker et al., 2012). The distal apical girdle of the G2 of *P. gibbesi* has also been described in species of Majoidea (Beninger et al., 1991; Neumann, 1996; Kienbaum et al., 2017) and Pinnotheridae (Becker et al., 2012) and may take part in sealing the ejaculatory canal within the G1.

3.4.2 | THE FEMALE REPRODUCTIVE SYSTEM

THE OVIDUCT ORIFICE

In *P. gibbesi* the oviduct does not enter the seminal receptacle directly, but leads into a separate cuticular duct connected to the vagina. This stands in contrast to all available descriptions of thoracotreme female reproductive systems. A corresponding condition has only been described in the Dorippidae MacLeay, 1838 (Hayer et al., 2016; Vehof et al., 2017). Dorippids are either considered as an early diverging lineage within the Heterotremata (Guinot et al., 2013; Tsang et al., 2014) or as a sister group of the remaining Eubrachyura (Jamieson et al., 1995; Ahyong et al., 2007). Whether a spatial separation of the seminal receptacle and the oviduct is the ancestral character state of the sperm storage system in Eubrachyura remains a controversial issue (see Hayer et al., 2016; Vehof et al., 2017). The different phylogenetic positions of Dorippidae and Percnidae render the situation even more problematic. No matter how the character changes are polarised - from a separation of oviduct and seminal receptacle to a direct connection of these structures, or vice versa – several homoplastic changes are always required.

The classification of seminal receptacles into dorsal and ventral types (sensu Diesel 1991) refers to the relative position of the oviduct/seminal receptacle-connection. McLay & López-Greco (2011) developed a hypothesis on the evolution of eubrachyuran seminal receptacles and consequences for fertilisation based on the different locations of oviduct orifices. The oviduct orifice of *P. gibbesi* is clearly ventral but since it does not join the seminal receptacle directly, the condition in this species does not fit into these concepts.

THE SECRETORY TISSUES OF OVIDUCT AND SEMINAL RECEPTACLE

Two different types of secretion occur in the seminal receptacle of *P. gibbesi*. The first type of secretion is produced by a dorsal glandular epithelium, which is very similar to that described in other thoracotreme species (Lautenschlager et al., 2010; de Souza et al., 2013; de Souza et al., 2017). The second type of secretion is produced by a holocrine transfer tissue (sensu Becker et al., 2011). In other thoracotremes, this tissue is found in the area of the connection between the oviduct and the seminal receptacle and in close proximity to the mono-layered glandular epithelium. It is made up of densely packed small cells which dissolve at the periphery of the tissue and are shed as secretion (Becker et al., 2011). Despite the unusual location of the oviduct orifice in *P. gibbesi*, it still leads into the same holocrine tissue as in other thoracotremes (Lee & Yamazaki, 1990; Lautenschlager et al., 2010; de Souza et al., 2013; Vehof et al., 2016; de Souza et al., 2017). In *P. gibbesi* the holocrine transfer tissue at the oviduct orifice is distant from the mono-layered glandular epithelium in the dorsal region

of the seminal receptacle. A sa result of these distinct locations and their histological differences, it is very likely that the holocrine tissue and the mono-layered glandular epithelium have a different origin within the reproductive system of thoracotreme females. The holocrine transfer tissue in *P. gibbesi* clearly originates from the oviduct.

The histological properties of the holocrine transfer tissue are consistent among thoracotremes (Lee & Yamazaki, 1990; Becker et al., 2011; de Souza et al., 2017) and very similar to the secretory tissues that line the seminal receptacles and contain the oviduct orifice in heterotremes (Beninger et al., 1993; Lanteigne et al., 1996; Sal Moyano et al., 2010; Zara et al., 2014; Antunes et al., 2016). Due to the shared histological characters and their association with the oviduct orifice, both tissues are likely homologous.

There are various hypotheses on the role of secretions within brachyuran seminal receptacles. They may support maintenance of the stored sperm (Johnson, 1980; Anilkumar et al., 1996; Becker et al., 2011), provide protection against bacteria (Jensen et al., 1996; Zara et al., 2014; Antunes et al., 2016), or promote the dehiscence of spermatophores (Diesel, 1989).

Eubrachyuran crabs are supposed to have trans-moult sperm retention because only the ventral part of the seminal receptacle is cuticle-lined, while the rest is secretory (McLay & Lopez-Greco, 2011; but see Hayer et al, 2016 and Vehof et al., 2017). The ratio of cuticle lining on the one hand, and secretory lining on the other hand can vary (Diesel, 1989; Jensen et al., 1996; Lautenschlager et al., 2010; Zara et al., 2014; Ewers-Saucedo et al., 2015; de Souza et al., 2017; Kienbaum et al., 2017). In *P. gibbesi* only a small area dorsally in the seminal receptacle is lined by glandular epithelium, while the main part of the seminal receptacle is lined by cuticle. The degree of cuticle lining in the seminal receptacle of *P. gibbesi* is therefore relatively high compared with other eubrachyurans (Lautenschlager et al., 2010; Pardo et al., 2013; Ewers-Saucedo et al., 2015; Hayer et al., 2015).

The unusual position of the oviduct connection in *P. gibbesi* has also implications relating to copulation and fertilisation. One important aspect is that oocytes are unlikely to enter the seminal receptacle and encounter spermatozoa therein at oviposition. Hence, the seminal receptacle is still the main sperm storage structure but not the site of fertilisation in *P. gibbesi* where the initiation of fertilisation is likely to occur in the relatively long vagina. The extensive musculature around the seminal receptacle of *P. gibbesi* may enable the release of spermatozoa into the vagina where they become mixed with the oocytes during oviposition.

THE BURSA

Another noteworthy character in the female reproductive system of *P. gibbesi* is the presence of an accessory sperm storage structure, the bursa, at the junction of seminal receptacle, cuticular duct and vagina. Bursae have only been described in very few heterotreme species, e.g. *Metacarcinus*

magister (Dana 1852): Jensen et al. (1996); *Limnopilos naiyanetri* Chuang & Ng 1991: Klaus et al. (2014); *Dorippe sinica* Chen 1980, *Dorippe quadridens* (Fabricius 1793): Vehof et al. (2017).

Interestingly, in all species investigated, the bursae were situated on the medial side of the seminal receptacle. While the bursae of *M. magister* (Jensen et al., 1996) is large in relation to the seminal receptacle, the bursae of dorippids (Vehof et al., 2017) and the herein studied *P. gibbesi* are rather small. The bursae are collapsed when they are empty and have a bulbous saclike appearance when filled with sperm. Only in dorippids, are bursae so strongly cuticularised that they keep their shape even when they are not filled with sperm (Vehof et al., 2017). Bursae were discussed in terms of sperm competition (Jensen et al., 1996; Jensen & Bentzen, 2012) and cryptic female choice (Klaus et al., 2014). Jensen & Bentzen (2012) propose that females discretely store male sperm and control whether the ejaculate enters the seminal receptacle (and will be used for fertilisation) or the bursa (and won`t be used for fertilisation). In species where multiple matings and mate guarding occur, bursae may serve a female strategy to "discard" sperm from some mates into the bursa, but still benefit from the protection provided through mate guarding.

Because the bursa is lined by cuticle instead of secretory tissue, it seems unlikely that sperm is viable for a long time and its content is supposedly shed during moult (Jensen et al., 1996). In *P. gibbesi* the opening of the bursa is in close proximity to the cuticular duct (including the oviduct orifice) and the vagina. Hence, it is possible that a supply of recently stored sperm is used for fertilisation. In the studied specimens of *P. gibbesi* only one female had filled bursae, whereas all seminal receptacles were filled. Whether this was due to the timing of sampling of specimens (which might have recently moulted) or whether the bursae in *P. gibbesi* are not routinely used for sperm storage and insemination remains unknown. The role of the bursa and whether stored spermatozoa are used for fertilisation, can only be revealed by paternity tests similar to the ones conducted by Jensen & Bentzen (2012).

3.5 | CONCLUSIONS

While the males of *P. gibbesi* show a copulatory system which is characteristic for Thoracotremata and similar to that of the grapsoids studied in this respect, the female reproductive system exhibits a novel combination of morphological characters and reveals a higher diversity of reproductive structures in thoracotremes as previously assumed. The connection of the oviduct through a separate cuticular duct joining the vagina and the presence of a bursa are both characters which have only been known from Heterotremata. There is however still a great lack of knowledge on the reproductive systems of Thoracotremata and no detailed studies of species closely related to *P. gibbesi* are available for comparison. In order to conclusively evaluate the condition of the oviduct orifice and the significance of the bursa as a phylogenetic character, more data are required. The bursa has previously been regarded as an apomorphy of a few species of Eubrachyura and was mostly

discussed in relation to cryptic female choice (Jensen & Bentzen, 2012; Klaus et al., 2014). However, with more studies emerging that describe bursae as accessory (Jensen et al., 1996; Klaus et al., 2014) or sole sperm storage structures (Vehof et al., 2017, 2018), the question arises whether those bursae are actually homologous and may represent the primary sperm storage structures of Eubrachyura. Future studies should focus on additional representatives closely related to *P. gibbesi* or belonging to putatively early diverging lineages of Thoracotremata in order to better understand the plesiomorphic character states in the sperm storage systems within this group. Special emphasis should thereby lie on the condition of the oviduct orifice, the position and histological characteristics of glandular tissues and epithelia and the incidence of bursae.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

For all citations provided, please see the concatenated reference list at the end of this thesis.

4 | THE REPRODUCTIVE SYSTEM OF *LIMNOPILOS NAIYANETRI* INDICATES A THORACOTREME AFFILIATION OF HYMENOSOMATIDAE (DECAPODA, EUBRACHYURA)

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ABSTRACT

The eubrachyuran Hymenosomatoidea is widely distributed in tropical and subtropical regions ranging from marine to freshwater habitats. Even though the biology of this taxon has been studied to some extent, its phylogenetic relationships are not resolved. Based on different morphological characters, some authors suggested a close affinity of hymenosomatid crabs to heterotremes. However, many of these characters are ambiguous, and the few molecular studies did not provide convincing solutions either. To address this issue, we studied the reproductive system of the hymenosomatid freshwater species *Limnopilos naiyanetri* Chuang and Ng, 1991 using histology and scanning electron microscopy. The females show the characteristic organization of the paired eubrachyuran reproductive system. Additionally, a bursa (an accessory sperm storing cuticle cavity) is present. The male copulatory system is characterized by paired long first and short second gonopods, and a pair of sternal gonopores equipped with a penis. Both, the female and male reproductive organs reveal a number of similarities to thoracotreme crabs. The seminal receptacle is lined by a very thin cuticle and by a mono-layered glandular epithelium. The male gonopods and the sternal genital opening also resemble the thoracotreme condition. Thus, our results indicate that Hymenosomatidae are most likely part of the Thoracotremata.

KEYWORDS: seminal receptacle, glandular epithelium, expandable cuticle, bursa, gonopods, sternal gonopore

4.1 | INTRODUCTION

The Hymenosomatidae consists of more than 100 described species (Guinot & Richer de Forges, 1997; Ng et al., 2008). It is one of the less well known eubrachyuran groups (Lucas & Davie, 1982; Chuang & Ng, 1994). Hymenosomatids are widely distributed in tropical and subtropical zones of the Indo-Pacific region (Lucas, 1980; Lucas & Davie, 1982; Feldmann & McLay, 1993; Ng & Chuang, 1996; Teske et al., 2009; Poore, 2010). Yet, some species also occur in South Africa (Teske et al., 2007; Teske et al., 2009) and the Pacific coast of America (Vinuesa & Ferrari, 2008). Hymenosomatids live in a wide variety of habitats, ranging from marine to freshwater and semi-terrestrial (Walker, 1969). They show a number of unique morphological and developmental characters such as a carapace encircled by a furrow – the hymenosomian groove (Guinot & Richer de Forges, 1997), a pleon that consists of maximally five segments and the telson forming a pleotelson (Lucas, 1980; Guinot, 2011). Other noteworthy characters are the generally abbreviated larval development and the absence of a megalopa (Guinot & Richer de Forges, 1997). Some freshwater species even show direct development (Lucas, 1971). Furthermore, their spermatozoal morphology seems to be unique (Richer de Forges et al., 1997).

The phylogenetic position of Hymenosomatidae is puzzling and not fully resolved. Based on some morphological characters, they have been considered as close relatives of either Majoidea (Chuang & Ng, 1994) or Inachoididae (Guinot & Richer de Forges, 1997). A molecular analysis resolved them as sister group to Dorippidae (Ahyong et al., 2007). Hence, the prevailing idea is that they belong to the heterotreme crabs, i. e. those eubrachyurans that are characterized by a coxal position of male gonopores (Guinot, 1977). However, the male sternal gonopore opening and the uniformly short second gonopod, both present in hymenosomatids, are characters associated with thoracotremes (Guinot, 1977; McLay & Becker, 2015). Yet, Guinot argued in a series of articles that the sternal male gonopores of Thoracotremata and Hymenosomatidae are convergences (Guinot & Richer de Forges, 1997; Guinot, 2011; Guinot et al., 2013).

Limnopilos naiyanetri Chuang and Ng, 1991 is a small-sized hymenosomatid crab, living in freshwater habitats in Thailand (Chuang & Ng, 1994). The female reproductive system of *L. naiyanetri* has been investigated by Klaus et al. (2014). However, these authors put the main focus on the biological function of the bursa, whereas the description of the seminal receptacle remained somewhat vague (Klaus et al., 2014). Therefore, with the increasing number of reported bursae in eubrachyuran species and with representatives of dorippoids (Vehof et al., 2017, 2018) as well as majoids (Kienbaum et al., 2017) studied, it seems plausible to re-examine the reproductive system of *L. naiyanetri* in detail with the goal to evaluate characters in order to resolve their phylogenetic position.

4.2 | MATERIAL AND METHODS

4.2.1 | MATERIAL

Five female and two male specimens of *L. naiyanetri* (carapace width 5 – 7 mm) were obtained from commercial vendors (www.interaquaristik.de).

4.2.2 | HISTOLOGY

For the histological analyses, female specimens were cold-anaesthetized in a freezer at –18 °C for 2 minutes. Whole specimens were preserved either in Bouin's solution or in "Susa Heidenhain" (MORPHISTO Evolutionsforschung und Anwendung GmbH, Frankfurt am Main, Germany) for 72 h. For decalcification, specimens were treated in 10% Ethylenediaminetetraacetic acid (EDTA) for 48 h. Specimens were then dehydrated through a series of increasing ethanol concentrations and infiltrated (Shandon Hypercenter XP, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and embedded with paraffin. Sections were prepared at 6–8 µm using a Leica RM2255 rotary microtome (Leica Microsystems GmbH, Wetzlar, Germany). All histological sections were stained with the trichromatic Masson-Goldner "light green" (MORPHISTO GmbH, Frankfurt am Main, Germany).

4.2.3 | SCANNING ELECTRON MICROSCOPY (SEM)

The dissected gonopods of the male specimen were manually cleaned using an eyelash attached to a glass pipette tip. The first and second gonopods were dehydrated through an ascending series of ethanol, critical point dried (Bal-Tec CPD 030, Balzers, Liechtenstein) and sputter coated with a gold layer (Bal-Tec SCD 005, Balzers, Liechtenstein). The micrographs were taken using a LEO (Zeiss) 1430 scanning electron microscope (Carl Zeiss Nano Technology Systems GmbH, Oberkochen, Germany).

4.2.4 | PHOTOGRAPHY AND IMAGE PROCESSING

Histological serial sections were viewed and photographed using an Axioskop 2 stereo microscope equipped with an Axio Cam HRc camera using AxioVision 4.3 (Carl Zeiss Vision GmbH). *In situ* multi-scan images of the male copulatory system and the female vulva were taken with a Keyence VHX1000 digital microscope (Keyence, Ōsaka, Japan).
All images were processed and assembled in figure plates using Adobe Photoshop (Adobe Systems Software, San Jose, USA), Corel Draw X6 and Corel Photopaint X6 (Corel, Ottawa, Canada).

4.3 | RESULTS

4.3.1 | THE FEMALE REPRODUCTIVE SYSTEM

The female reproductive system of *L. naiyanetri* consists of paired ovaries that are connected to the likewise paired oviducts. The oviducts are ventrally connected to the seminal receptacle at the transition to the vagina (Fig. 4.1). The sperm is stored in the seminal receptacles and accessory sperm storage structures, the bursae. The seminal receptacle, the oviduct, the bursa, and the vagina share a joint connection in very close proximity to each other (Figs. 4.1, 4.2B).

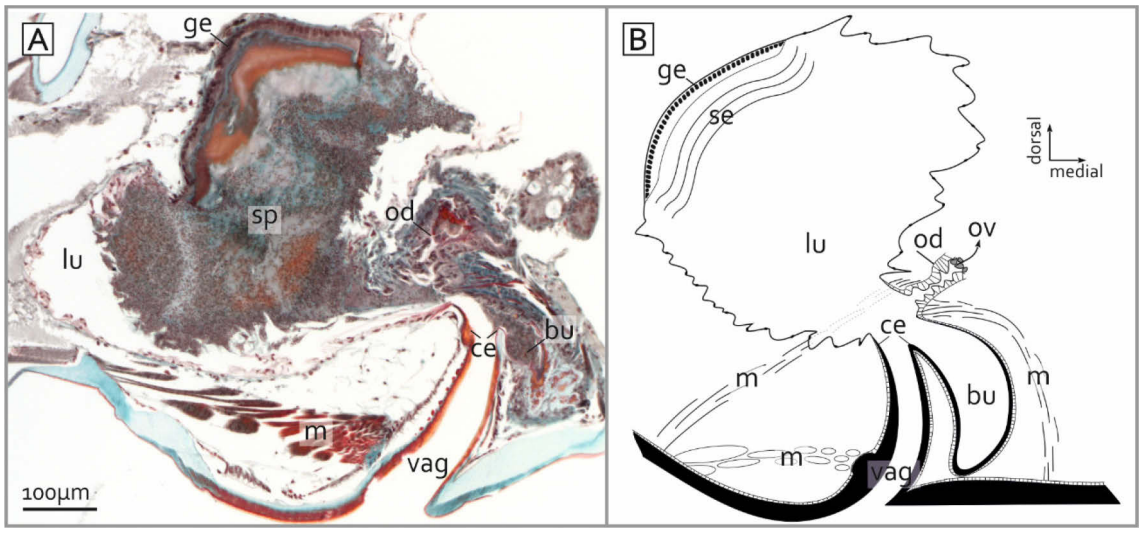

Fig. 4.1 Overview of the female reproductive system of *Limnopilos naiyanetri*. (A) Histological section; (B) Schematic drawing. For explanations see text.
Abbreviations: bu – bursa, ce – cuticle epithelium, ge – glandular epithelium, lu – lumen, m – muscle, od – oviduct, ov – ovary, se – secretion, sp – sperm mass, vag – vagina

Different stages of oocyte proliferation are present in the ovary. Vitellogenic oocytes can be found in close proximity to the oviduct (Fig 4.2A). The epithelium of the oviduct forms a short duct. Its transition into the epithelium of the seminal receptacle is smooth. In the immediate vicinity of the transition between the oviduct and the seminal receptacle a conglomeration of secretion is present (Fig. 4.2A-B). This secretion is indirect evidence for the existence of a secretory tissue, the "holocrine transfer tissue" (sensu Becker et al., 2011). In some females, sperm can be found adjacent to these secretions (Fig. 4.2A).

The bursa is situated at the antero-medial side of the seminal receptacle and is completely lined by cuticle (Fig. 4.2B-D). In all females both bursae were filled with sperm. Those sperm masses were often continuous with the sperm mass in the seminal receptacles (Fig. 4.2C). Only free spermatozoa but no spermatophores were present in the seminal receptacle and bursa. The cuticle of the bursa

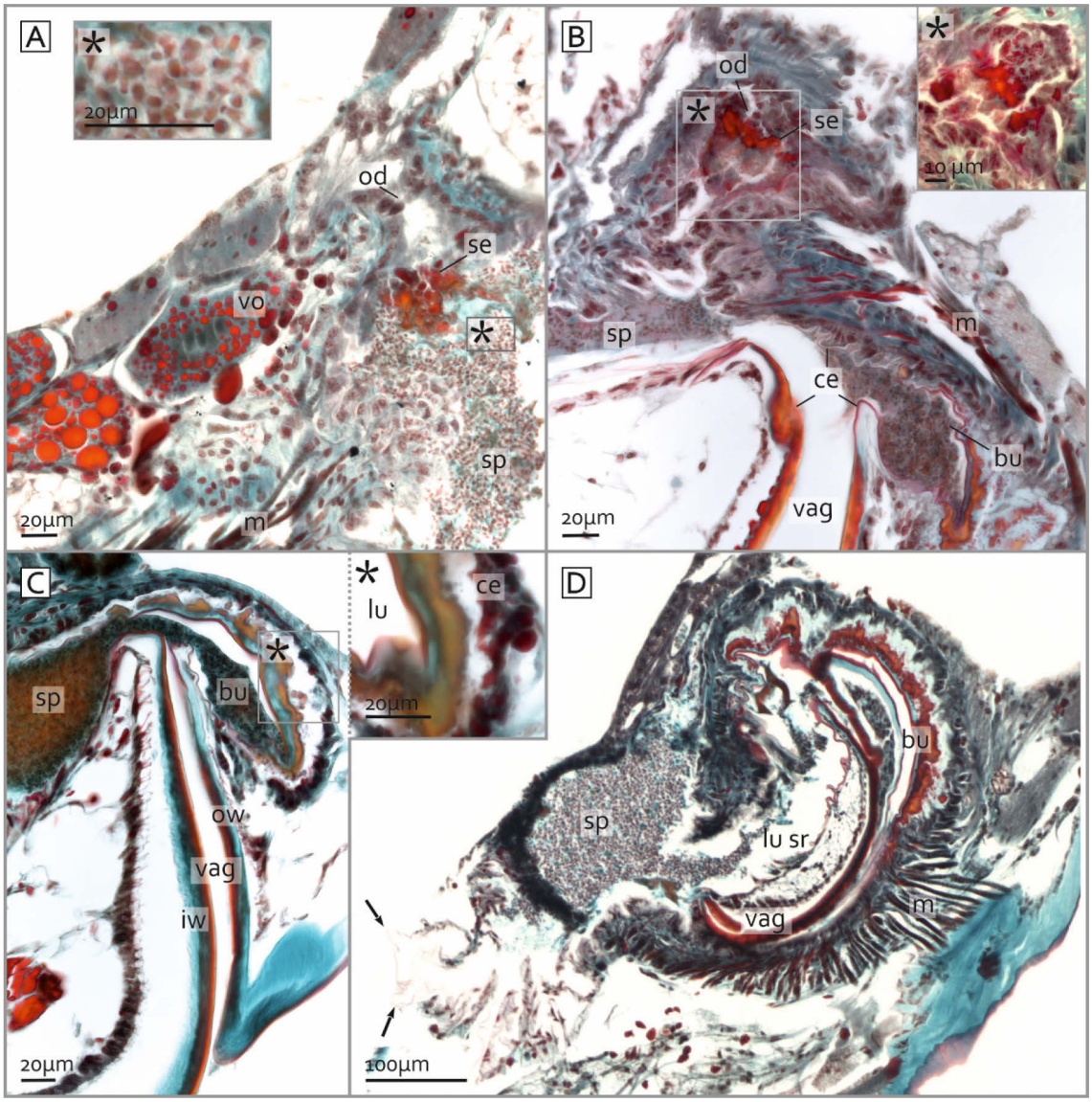

Fig. 4.2 Histological sections of the oviduct, bursa and vagina of female *Limnopilos naiyanetri*. (A) Parts of the ovary filled with vitellogenic oocytes and the oviduct transition into the seminal receptacle with secretions into the lumen. (*= Sperm adjacent to the released secretions.) (B) The oviduct orifice is situated in very close proximity to the bursa and vagina. Both are lined by cuticle epithelium. The muscle runs from the sternum to the area between the oviduct and bursa (* = detail of the secretory tissue and secretion at the oviduct orifice). (C) The vagina and bursa. (* = detail of the bursa showing different cuticle layers through different staining properties in Masson-Goldners trichrome.) (D) Cross section through the vagina and parts of the seminal receptacle (The arrows point to the very thin epithelium). Muscle fibers connect the outer vagina wall to the sternum.

Abbreviations: bu – bursa, ce – cuticle epithelium, iw – inner vagina wall, lu – lumen, m – muscle, od – oviduct, ow – outer vagina wall, se – secretion, sp – sperm mass, sr – seminal receptacle, vag – vagina, vo – vitellogenic oocyte

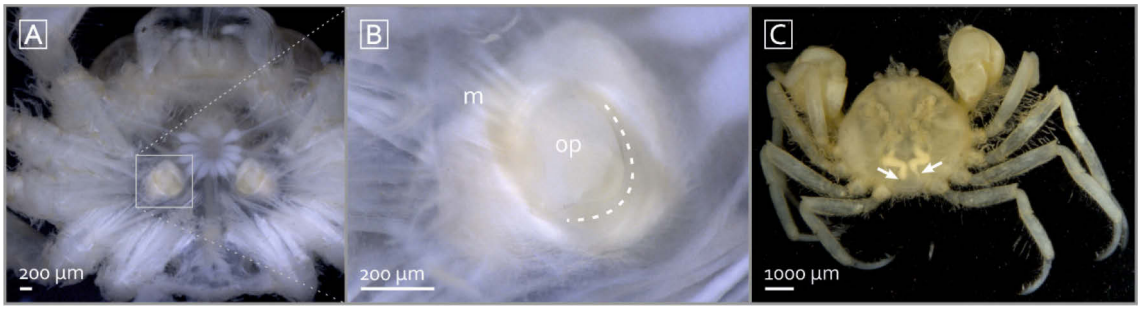

Fig. 4.3 The position of the gonopores of female and male *Limnopilos naiyanetri* (Keyence digital microscopy). (A) The sternal position of the female vulva, ventral view. (B) The vulva of the females is closed by an operculum. A large muscle is attached to the concave vagina wall (visible through the transparent carapace). (C) The male reproductive system is shown through the transparent carapace. Arrows indicate the sternal position of the external extension of the ejaculatory duct (= penis). The ejaculatory ducts do not project to the coxae.
Abbreviations: m – muscle, op – operculum

appears different compared with the cuticle of the vagina in showing an additional orange-staining layer in Masson-Goldners trichrome (see detail of Fig 4.2C).

The vagina is crescent shaped (Fig. 4.2D) with the vulva being covered by an operculum (Fig. 4.3A-B). A relatively large muscle connects the inner concave vagina wall to the sternum (Figs. 4.1, 4.3B). Additionally, two smaller muscles (with only few muscle fibers) are present. One connects the area between the oviduct and the bursa with the sternum on the medial side. The second muscle also connects the sternum with a region between the oviduct and seminal receptacle (Fig. 4.1B).

Ventrally, the cuticle lining of the vagina and bursa transits smoothly into the cuticle of the seminal receptacle. The underlying epithelium shows irregularly distributed cell nuclei and is covered by a very thin extracellular matrix. It appears to be cuticle due to the usual arrangement in the ventral area of a eubrachyuran seminal receptacle, the smooth transition from the vagina and bursa, and the absence of any secretion in this region. Dependent on the amount of sperm filling it is either strongly folded (few sperm) or stretched (more sperm) (Fig. 4.4A-D).

The second type of epithelium is glandular. It is a mono-layered columnar epithelium consisting of regularly shaped cells with large, basally located nuclei situated dorso-laterally in the receptacle. Its secretions are released in layers into the seminal receptacle lumen (Figs. 4.1A; 4.4A-B).

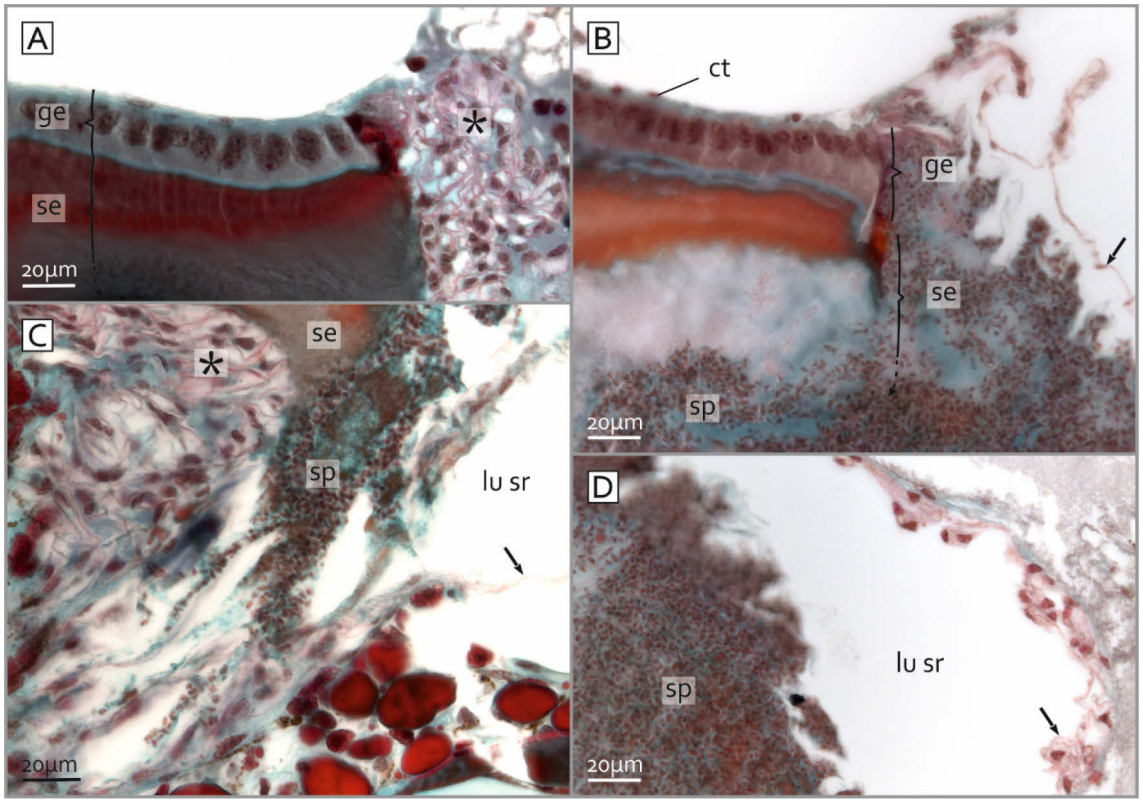

Fig. 4.4 Histological sections of the seminal receptacle of female *Limnopilos naiyanetri*. (A) The glandular epithelium in the dorso-lateral area of the seminal receptacle and the transition into the strongly intertwined second type of epithelium (see *). (B) The glandular epithelium and the transition into the elongated second type of epithelium (see arrow). Secretions appear in layers and the seminal receptacle is filled with free spermatozoa. (C) The intertwined epithelium of the seminal receptacle (see *) can be elongated significantly (see arrow), depending on the amount of sperm present in the seminal receptacle. (D) The nuclei are randomly distributed within the very thin epithelium (see arrow) of the seminal receptacle.
Abbreviations: ct – connective tissue, ge – glandular epithelium, lu sr – lumen of seminal receptacle, se – secretion, sp – sperm mass

4.3.2 | THE MALE COPULATORY SYSTEM

The paired male copulatory system consists of the first gonopods (G1), the second gonopods (G2), and the penis (Fig.4.5A). The G1 is stout and curved in an S-shape. Its tip consists of two teeth (Fig.4.5A, B see *) and an additional lobe (Fig.4.5A, B see °). The larger triangular tooth lies adjacent to the lobe and a smaller sharp tooth is present on the opposite side of the ejaculatory canal opening (Fig. 4.5A-B). A row of pappose setae runs along the distal part of its medial surface.

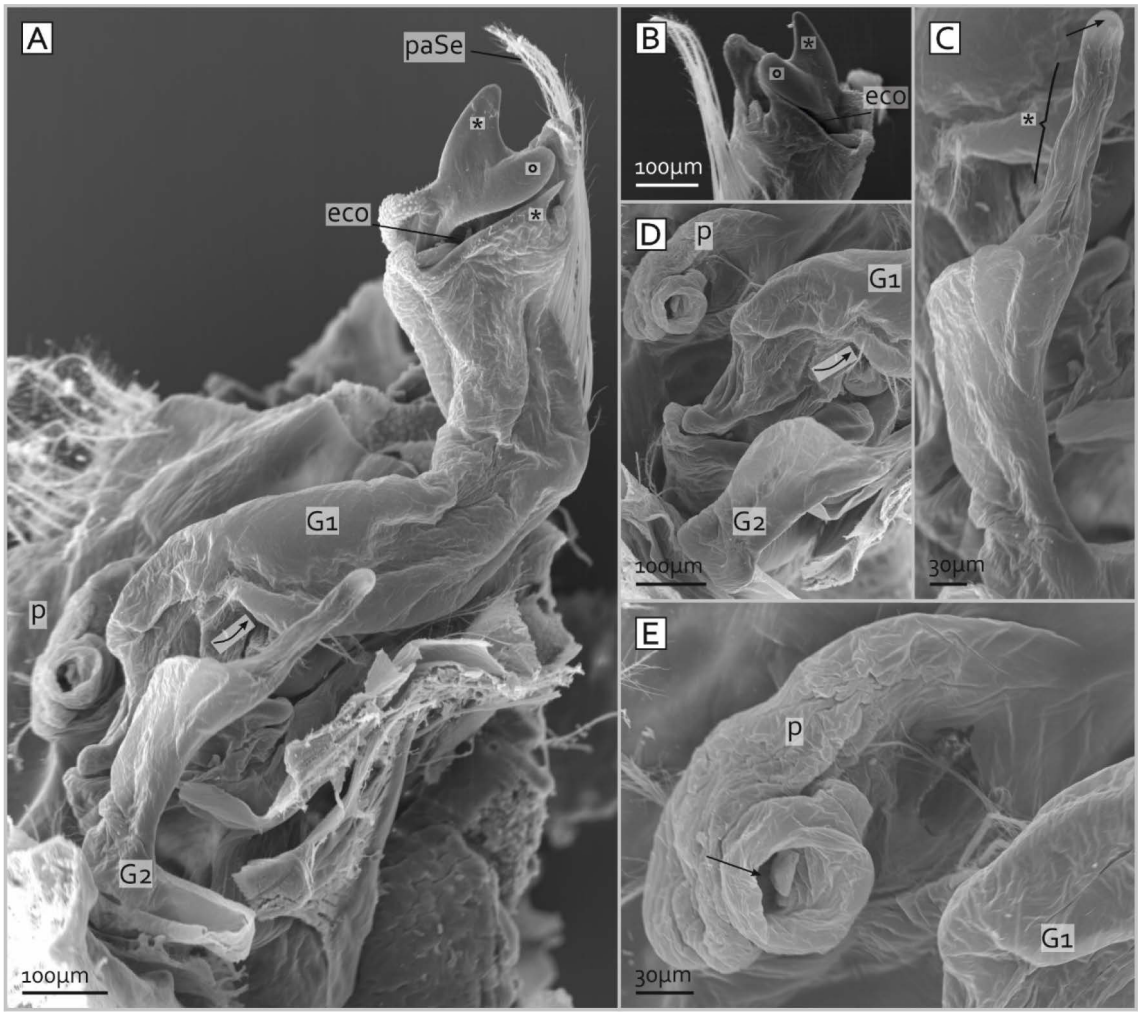

Fig. 4.5 Scanning electron microscopy of the gonopods and penis of male *Limnopilos naiyanetri*. (A) Overview. The arrow indicates the insertion site for the G2. On the tip of the G1, the cuticle teeth (*) and the cuticle lobe (°) are present. (B) The tip of the G1. The ejaculatory canal opening is adjoined by two cuticle teeth (*) and one cuticle lobe (°). (C) The second gonopod is very small and delicate. It has a round tip (see arrow) and many cuticle folds along the distal part (see *). (D) The insertion site for the G2 can be found in the lower third if the first gonopod (see arrow). (E) The penis is a small cuticle projection with a round opening (see arrow).
Abbreviations: eco – ejaculatory canal opening, G1 – first gonopod, G2 – second gonopod, p – penis, paSe – pappose setae

The G2 is small and delicate and covered with a thin cuticle. Its tip is round and there are no apparent additional structures present. Its surface is smooth without any setae, but with folds along the distal part (Fig. 4.5C).

The penis emerges from the sternum in the form of a papilla with a round opening (Figs. 4.3C, 4.5D-E).

4.4 | DISCUSSION

4.4.1 | THE MORPHOLOGY OF THE FEMALE REPRODUCTIVE SYSTEM

OVIDUCT ORIFICE AND THE TISSUE OF THE SEMINAL RECEPTACLE

The variety of the oviduct orifice position of eubrachyurans has been thoroughly reviewed by McLay & López-Greco (2011). The oviduct orifice always connects to a multi-layered secretory tissue that lines the seminal receptacle. In heterotremes, the position of the oviduct orifice varies to a certain degree (McLay & López-Greco, 2011; McLay & Becker, 2015). Irrespective of this position, in many heterotreme species the orifice is situated directly at the transition of the cuticular and secretory areas of the seminal receptacle ("ventral-type seminal receptacle" sensu (Diesel, 1991; de Souza et al., 2017). In contrast to this, the investigated representatives of portunoids (Johnson, 1980; Zara et al., 2014; Pardo et al., 2017), some pilumnoids (Diesel, 1991; McLay & López-Greco, 2011), platyxanthids (Farias et al., 2017) and the xanthoid *Lybia tessellata* (Latreille in Milbert, 1812) pers. obs.), the oviduct orifice connects to the seminal receptacle in an area that is lined by the multi-layered secretory tissue but not in immediate proximity to the cuticle-lined area and the vagina ("dorsal-type seminal receptacle" sensu Diesel, 1991). In Thoracotremata, a multi-layered secretory tissue, ("holocrine transfer tissue" sensu Becker et al., 2011), is restricted to the oviduct orifice (Lee & Yamazaki, 1990; López-Greco et al., 2009; Lautenschlager et al., 2010; Becker et al., 2011; Vehof et al., 2016; de Souza et al., 2017; Kienbaum et al., 2018a). Due to their histological similarities, it is very likely that the multi-layered secretory tissue of the seminal receptacle of heterotremes is homologous to the holocrine transfer tissue in thoracotremes (McLay & Sal Moyano, 2016; Kienbaum et al., 2018a). In Thoracotremata, the holocrine transfer tissue is either positioned adjacent to a mono-layered glandular epithelium or cuticle. In the latter case the mono-layered glandular epithelium of thoracotremes, has no direct contact to the multi-layered secretory tissue at the oviduct orifice.

In *Limnopilos naiyanetri*, the presence of secretions at the oviduct orifice indicates the occurrence of secretory tissue in this region. Furthermore, the restriction of this secretory tissue to the oviduct orifice suggests that this is the holocrine transfer tissue. In addition, we found a dorsal area with a mono-layered glandular epithelium.

The largest part of the wall of the seminal receptacle consists of a thin epithelium equipped with a delicate cuticle. This cuticle has an enormous ability to expand, depending on the amount of sperm masses and fluids in the seminal receptacle. Such an enormous expandability has been described for the seminal receptacle of the heterotreme plathyxanthid crab *Danielethus crenulatus* (A. Milne-Edwards, 1879) by Farias et al. (2017). However, in *D. crenulatu*s this is related to the secretory tissue and not to the cuticle-lined area as in *L. naiyanetri*.

The muscles that connect two different parts around the oviduct orifice with the sternum possibly increase the lumen of the seminal receptacle in this region. This might facilitate the ovulation by providing space and some sort of direction for the oocytes by adjusting this part of the seminal receptacle to the vagina opening.

BURSAE, ACCESSORY SPERM STORAGE STRUCTURES

Bursae are accessory sperm storage structures that are primarily known from heterotreme species such as *Metacarcinus magister* (Dana, 1852): Jensen et al. (1996); *Dorippe sinica* Chen, 1980, and *Dorippe quadridens* (Fabricius, 1793): Vehof et al. (2017). Thus far, the only known thoracotreme species with bursae is *Percnon gibbesi* (H. Milne Edwards, 1853) (Kienbaum et al., 2018a). As in the other species, the bursae in *L. naiyanetri* are situated at the antero-medial side of the seminal receptacles. Some interesting inferences arise regarding its position between the oviduct orifice and the vagina:

In *L. naiyanetri* all bursae are more or less filled with sperm independent of the degree of filling of the seminal receptacle. Jensen & Bentzen (2012) argue that females actively discriminate whether male sperm enters the seminal receptacle or the bursa and thereby control which sperm is used for fertilization. Since sperm that has been transferred by the gonopods would enter the seminal receptacles and the bursae equally, a function of the bursa in cryptic female choice as suggested by Klaus et al. (2014) seems unlikely in *L. naiyanetri*.

Moreover, our results contradict the conclusion by Klaus et al. (2014) that "sperm stored in bursa is distant from the site of fertilization". In fact, the sperm within the bursa is closer to the oviduct orifice than the sperm in the dorsal part of the seminal receptacle. Nonetheless, due to its cuticular lining, a secretory activity is not present in the bursa. Thus, the sperm is not nourished and is shed with moulting (Jensen et al., 1996). Hymenosomatidae are assumed to have a final moult (Guinot et al., 2013), which would argue against the loss of the sperm from the bursa. Still, the difference between sperm stored in the seminal receptacle and that stored in the bursa, might lie in long or short term usage.

The vagina is of the concave type (sensu Hartnoll, 1968). The large muscle connected to the inner vagina wall enables the female to increase the vagina lumen. This might facilitate the insertion of the gonopod during copulation.

In contrast to Klaus et al. (2014), who described a "non-sclerotized dorsal part", our results show that the vagina is completely lined by cuticle. This cuticle transits smoothly into the cuticle of the ventral seminal receptacle area.

Klaus et al. (2014) interpreted the tooth on the gonopod tip as a "device to facilitate forced copulations" or to "harm the female genital tract, hampering subsequent copulations". Both

hypotheses seem unlikely. The damage of the vagina or vulva would not only compromise the general fitness of the females but also oviposition and therefore reduce the reproductive success.

4.4.2 | PHYLOGENETIC IMPLICATIONS

HETEROTREME OR THORACOTREME, THE PROBLEMATIC PHYLOGENETIC POSITION OF HYMENOSOMATIDAE

The phylogenetic relationships of Hymenosomatidae among Brachyura have been controversial for a long time. Some authors favoured a position within the heterotreme crabs, namely in close affinity to Majoidea (Rathbun, 1925; Guinot & Richer de Forges, 1997; Richer de Forges et al., 1997; Guinot & Bouchard, 1998) whereas others suggested a thoracotreme affinity, close to the Pinnotheridae (Alcock, 1900; Gurney, 1938; Garth, 1958; Lucas, 1971; McLay, 1988). Likewise, recent phylogenetic analyses based on molecular data led to contradictory results. Ahyong et al. (2007) resolved hymenosomatids as close relatives of Dorippoidea within heterotremes, whereas the analysis of Teske et al. (2009) resulted in a close relationship to Potamidae. Interestingly enough, the latter have been recently discussed as closely related or even as sister group to Thoracotremata (Shen et al., 2013; Basso et al., 2017; Tang et al., 2017; Yuhui et al., 2017).

Guinot (2011), Guinot et al. (2013), and Davie et al. (2015b) discussed the contradicting data concerning the hymenosomatid position within the Eubrachyura. The hymenosomatids show a number of characters such as unique spermatozoal characters, larval morphology, and abbreviated larval development, the lack of a megalopa, incomplete or absent orbits, and the "hymenosomian groove" that can neither be assigned to heterotremes nor thoracotremes. Guinot et al. (2013) interpreted these characters as ancient traits and concluded that hymenosomatids are an old heterotreme group with a Gondwana origin. Based on similarities of the axial skeleton and some external characters Guinot (2011) and Guinot et al. (2013) concluded that hymenosomatids are close relatives of dorippoids. Since this agreed with the molecular analysis of Ahyong et al. (2007), the authors suggested that the relationship of Hymenosomatoidea and Dorippoidea "appear unambiguous" (Guinot et al., 2013, p.221). In addition, Davie et al. (2015b, p. 946) even stated that Hymenosomatidae has an "incontestable heterotreme status".

However, the situation is not as clear as claimed by these authors. For instance, the similarities in the axial skeleton as stated by Guinot et al. (2013) might not be complex correspondences but simply relate to the overall round shape of the sternum found in Dorippidae and Hymenosomatidae. Moreover, Guinot et al. (2013) partly argue with parallel trends in the evolutionary transformation such as the shape of the first male gonopods of dorippoids and hymenosomatids. Trends cannot be used as proper characters. On the other hand, characteristic features of the Hymenosomatidae that are different or absent in the Dorippidae such as the sternal male gonopores were regarded as

convergences (Guinot et al., 2013). In contrast to the view of Guinot et al. (2013), the characters that are unique to Hymenosomatidae appear to be autapomorphies, strongly corroborating hymenosomatid monophyly, rather than indications of the group being an ancestral (eu)brachyuran lineage.

THE REPRODUCTIVE SYSTEM AND ITS BEARING ON THE PHYLOGENETIC POSITION OF HYMENOSOMATIDAE

The female reproductive system of *L. naiyanetri* is characterised by the presence of a bursa, a dorsal mono-layered glandular epithelium, a holocrine transfer tissue in the seminal receptacle and a concave vagina. The male system shows a G1 that is longer than the G2, a reduced G2 lacking surface structures, and a sternal condition of the male gonopore. Some of these characters are not informative for the problem of the phylogenetic position of hymenosomatids. For instance, a bursa has been described for a number of heterotreme and thoracotreme crabs (see above; Kienbaum et al. 2018; Vehof et al. 2018). The same is true for a concave vagina and a G1 that is longer than the G2 (Beninger et al., 1991; Guinot et al., 2013; McLay & Becker, 2015; Ewers-Saucedo et al., 2016; Kienbaum et al., 2017; Vehof et al., 2018). However, several characters of the female and the male reproductive systems of *L. naiyanetri* suggest a thoracotreme affinity. In particular, female characters such as the mono-layered glandular epithelium in the dorsal area and the holocrine transfer tissue of the seminal receptacle correspond to the thoracotreme condition (Lee & Yamazaki, 1990; López-Greco et al., 2009; Becker et al., 2011; de Souza et al., 2017; Vehof et al., 2017). The secretions produced in the seminal receptacle of *L. naiyanetri* appear very similar to those described for the grapsoid *P. gibbesi* (Kienbaum et al., 2018a). In addition, the male characters like the G2 lacking surface structures and the sternal gonopore are clearly shared with thoracotreme males (McLay & Becker, 2015). In particular, there is no real reason to interpret the latter character as homoplasy to the thoracotreme condition as discussed by several authors (Jamieson & Tudge, 2000; Guinot, 2011; Guinot et al., 2013).

In contrast to this, the similarities to the reproductive organs of majoids and dorippoids are scarce. For example, in both groups the male gonopore is situated on the coxae and the G1 of dorippoids shows a different size relation to the G2 compared with *L. naiyanetri*. The female seminal receptacle of majoids is dorsally lined by the multi-layered secretory tissue (Kienbaum et al., 2017). The female reproductive systems of Dorippoidea show unique characters such as the cuticle valves at the oviduct orifice that are different from other eubrachyuran species (Hayer et al., 2016; Vehof et al., 2017, 2018). Furthermore, the ground pattern of dorippoids possessed most likely a bursa in combination with a cuticle lined seminal receptacle or even another type of sperm storage chambers (Vehof et al., 2018).

In summary, the reproductive system of *L. naiyanetri* reveals no indication for a close relationship of hymenosomatids to majoids or dorippoids or any other heterotreme group. Hence, in contrast to the conclusions of Guinot (2011), Guinot et al. (2013) and Davie et al. (2015b) our results suggest a thoracotreme affinity.

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REFERENCES

For all citations provided, please see the concatenated reference list at the end of this thesis.

5 | DISCUSSION

5.1 | THE COPULATORY SYSTEM OF MALE BRACHYURA

The evolutionary transformation of the brachyuran gonopods resulted in the unique and complex male copulatory system in which the penis, the tubular G1 and the G2 are used to accurately transfer sperm into the female reproductive system. Hereby, the G1 or the G2 act as intromittent organs when the sperm has been extruded by the penis into the ejaculatory canal of the G1. The diversification of the gonopods might be of use for phylogenetic investigations as suggested by different authors (Bauer, 1986; Beninger & Larocque, 1998; von Sternberg et al., 1999). However, most taxonomic research gives only (and restricted) information on the G1, omitting data on G2 morphology. Complete descriptions, including position of musculature, setae morphology and gonopod tegumental glands are scarce (Beninger et al., 1991; Minagawa, 1993; Kienbaum et al., 2017, 2018a: chapter 2, 3). Nevertheless, all these aspects provide important data to evaluate the use of the gonopod morphology for phylogenetic studies and to discuss topics such as functional morphology of the gonopods and related hypotheses on, for example, the transportation of the sperm (Beninger et al., 1991; Brandis et al., 1999; Becker et al., 2012). The copulatory system of the herein investigated majoid species *Mithraculus sculptus* (Lamarck, 1818) and *Stenorhynchus seticornis* (Herbst, 1788) (Kienbaum et al., 2017: chapter 2), as well as the grapsoid species *Percnon gibbesi* (H. Milne Edwards, 1853) (Kienbaum et al., 2018a: chapter 3) and the hymenosomatoid species *Limnopilos naiyanetri* Chuang and Ng, 1991 (Kienbaum et al., 2018b: chapter 4), will be compared with the existing data (Spalding, 1942; Diesel, 1989; Beninger et al., 1991; Minagawa, 1993; Neumann et al., 1996; Beninger & Laroque, 1998; Brandis et al., 1999; George, 2004; Lautenschlager et al., 2010; Sal Moyano et al., 2011; Becker et al., 2012; Vallina et al., 2014; Ewers-Saucedo et al., 2015, 2016; Vehof et al., 2018) and interpreted in relation with the mentioned issues.

5.1.1 | GONOPOD MORPHOLOGY – IT'S A MATTER OF FORM, RATHER THAN SIZE

PODOTREME GONOPODS

Bauer (1986) proposes that the degree of modification of appendages for sperm transfer can be used as a measure of the phylogenetic distance from the ancestral state and regards the biramous, natatory pleopods as the "primitive" form.

In most decapod species the gonopods are almost equally long. In the Dendrobranchiata both gonopods are biramous. The endopods of the G1 are modified and join to form a petasma and the G2 bears an additional process, the appendix masculine (Bauer, 1986, 1991). In the Caridea, both gonopods are biramous and the G2 is slightly longer than the G1 (Bauer, 1976). Andrews (1911)

investigated the sperm transfer in a cambarid species. Here both gonopods are modified for sperm transfer and only the G2 is biramous. The G2 is slightly longer than the G1.

Within the Anomala that represent a probable sister group to the Brachyura (Scholtz & Richter, 1995; Shen et al., 2013), the Diogenidae (Hess & Bauer, 2002) and the Galatheidae (Kronenberger et al., 2004) have been investigated. In the diogenid *Clibanarius vittatus* (Bosc, 1802), the G1 are completely reduced and the G2 as well as all other remaining pleopods are present only on the left side. The G2 is biramous and does not seem to be modified for sperm transfer (Hess & Bauer, 2002). In the galatheid *Galathea intermedia* Lilljeborg, 1851, both gonopods consist of three segments and the G2 is slightly longer and broader than the G1. Both gonopods are modified for sperm transfer (Kronenberger et al., 2004).

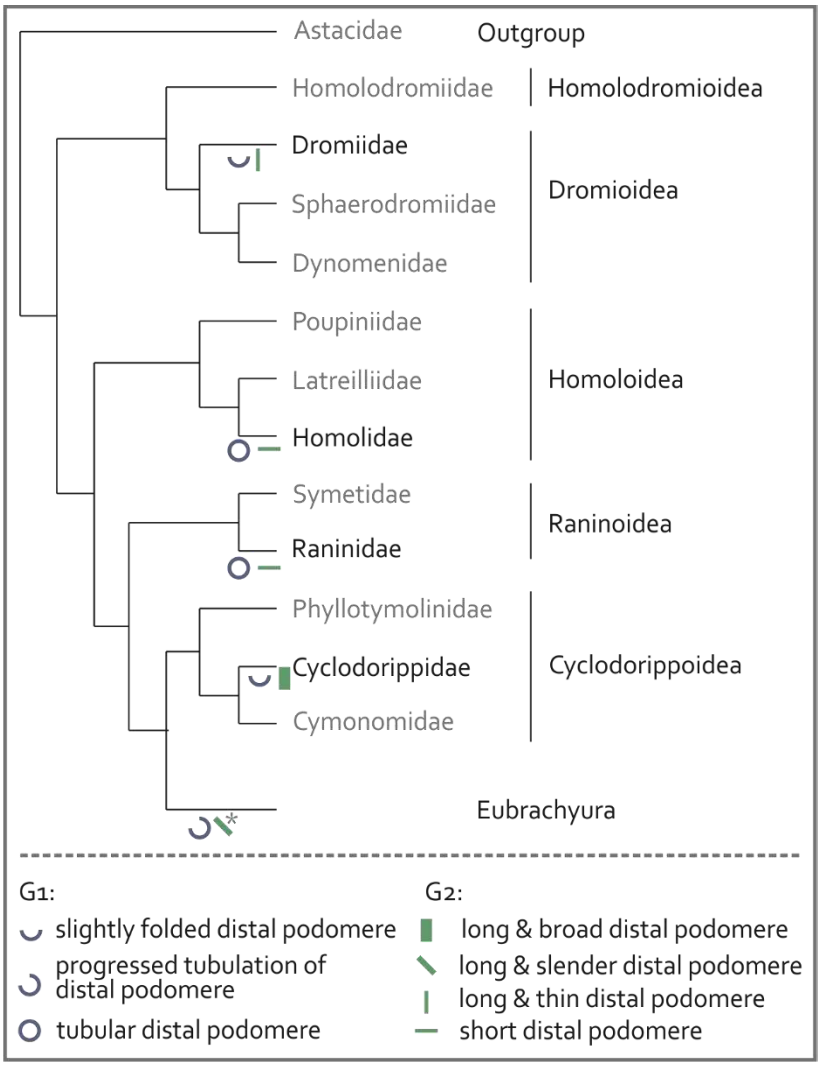


Fig. 5.1 One possible hypothesis about paraphyletic "Podotremata" modified from Karasawa et al. (2011). Gonopod character states of four podotreme species from four podotreme groups that are shown in Fig. 5.2. The G1 has a tubular distal podomere in Homolidae and Raninidae. In both groups, the G2 is short. In Dromiidae and Cyclodorippidae the G1 is not entirely folded. In these groups, the G2 is long. It is elongated and slender in Dromiidae and broad in Cyclodorippidae.
Please note that "long" and "short" are meant relatively to describe "the G2 is as long or longer than the G1" and "the G2 is shorter than the G1". * = the hypothetical character state at the base of the Eubrachyura might have been almost equally long gonopods.

Based on this data, the plesiomorphic state in podotreme males was probably characterised by almost equally long gonopods, each consisting of three podomeres. The distal podomere of the G1 started to slightly fold longitudinally, thereby forming a groove or channel. This is the case in the early diverging lineage of Dromiidae (Stephensen, 1946; Figs. 5.1, 5.2) but also in the taxon Cyclodorippidae that is being discussed as sister group to the Eubrachyura (Karasawa et al., 2011; Figs. 5.1, 5.2). In contrast to this, the tubulation of the G1 is progressed in Raninidae (Minagawa, 1993) and Homolidae (McLay & Becker 2015; Becker & Scholtz 2017; Fig. 5.1, 5.2). Thus, the degree of the gonopod transformation in "Podotremata" is not gradual. In accordance with the assumption of paraphyletic "Podotremata" (Spears et al., 1992; Brösing et al., 2006; Ahyong et al., 2007; Scholtz & McLay, 2009; Karasawa et al., 2011; Tsang et al., 2014) multiple transformations of the G1 within its constituting groups seem plausible.

The hypothesis of Guinot et al. (2013) of a long and thin G2 as plesiomorphic in podotreme and eubrachyuran crabs might need a careful reevaluation (Fig. 5.1). The long G2 in the early diverging Dromioidea could equally be interpreted as an apomorphy of this group. The same non-linear transformation as in the G1 can be assumed for the G2. It elongated and thinned in Dromiidae (Stephensen, 1946), broadened in Cyclodorippidae (data and personal communication from J. Vehof; Fig. 5.2) and became short and stouter in for example Raninidae (Minagawa, 1993) and Homolidae (McLay & Becker 2015; Fig. 5.2). Interestingly, the latter two groups have a G1 with a tubular distal podomere, a possible correlation that has also been discussed for heterotremes.

HETEROTREME GONOPODS

As in the paraphyletic podotremes, the plesiomorphic state in eubrachyuran males was probably characterised by almost equally long gonopods. The distal podomere of the G1 showed a progressing tubulation, while the distal podomere of the G2 was still relatively long and slender.

Gonopods of a species or group have so far only been classified according to the size relationship of the G1 and the G2. This resulted in two categories: Either the G1 is longer than the G2 or the G2 is longer than the G1 (Davie et al., 2015a; McLay & Becker, 2015). The distribution of these two categories within and between heterotreme groups is variable. Additionally, this size-based categorisation does not consider and incorporate the range of different gonopod shapes in heterotreme males (Fig. 5.2). Notably, in species with long G2, the forms of the G1 show some conformity. They possess a relatively broad proximal part and taper towards the distal tip in a slightly bending, conoid shape. The ejaculatory canal opening is terminal. On the other hand, the G1 of species with short G2 are more slender. The ejaculatory canal opening can be terminal (for example in Xanthidae, pers. obs.) or subterminal (for example in Majoidea, Kienbaum et al. 2017: chapter 2). These morphological differences can be explained by the space that is required for the G2. Since the

long G2 is positioned within the total length of the ejaculatory canal, it takes up more space than the short G2 that is only positioned within the proximal part of the G1. Therefore, a tendency towards a more slender form of the G1 is probably only possible in males with short G2. The elongated and slender gonopod forms of the herein investigated heterotreme species (Kienbaum et al. 2017: chapter 2) coincides with this Hypothesis. Becker et al. (2012) reason that in species with long G2, the tubulation of the G1 is incomplete resulting in an open suture of the ejaculatory canal to enable the insertion of the G2 laterally. Consequently, the advanced tubulation of the G1 would hamper the lateral insertion of a long G2 and might be closely correlated with its reduction in length.

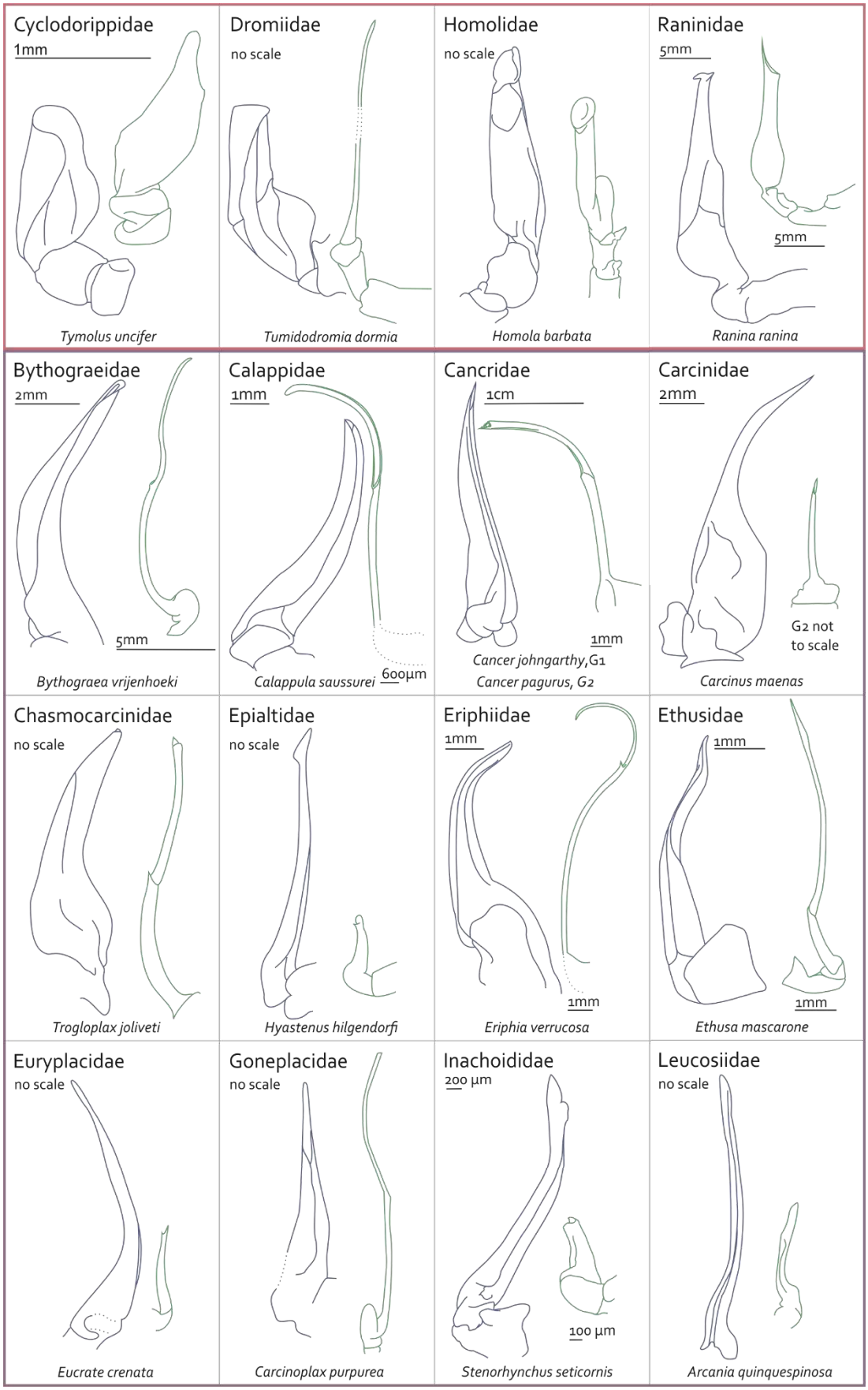
A comparison of the long G2 reveals an interesting similarity of their forms. The elongated distal podomere forms a "spoon-like structure" approximately half way along the podomere (sensu Brandis et al. 1999). The same structure is called "terminal joint" by Ewers-Saucedo et al. (2016) who studied gonopod morphology of Calappidae. From external morphological characters it remains unclear if these gonopods possess an additional joint, or whether this external morphology is the result of a fusion of two podomeres and the associated loss of the actual joint, or equally possible, if this is only a special external morphological character of one podomere. Therefore, it is more adequate to refrain from the use of "joint" in this context but to use the term "spoon-like structure" of Brandis et al. (1999).

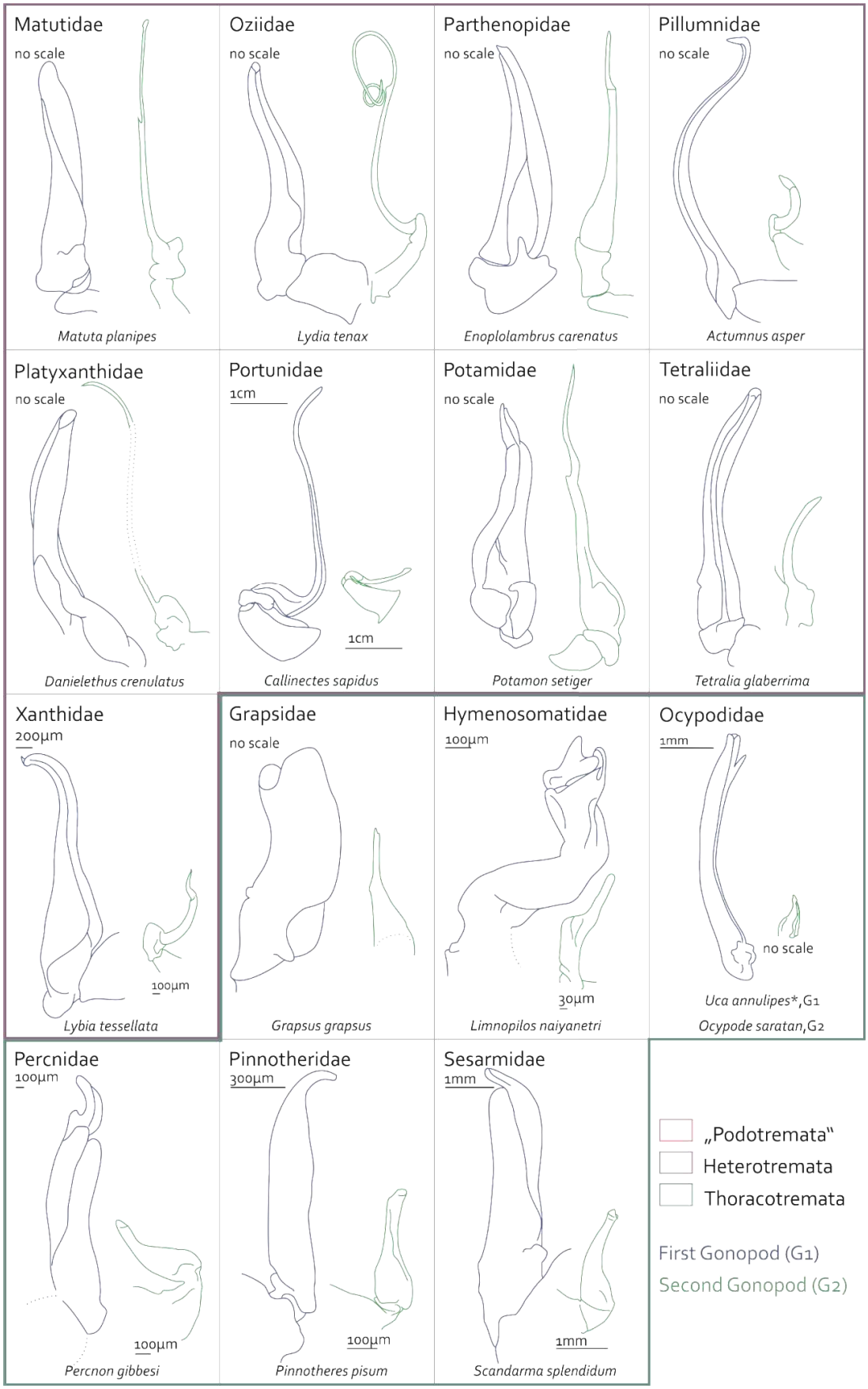
Two evolutionary transformation series from long into short G2 are feasible. Both of these transformations could have happened multiple times. The G2 could have undergone the transformation from the plesiomorphic state. This would involve the size reduction of the G2 into a shorter form, as is, for instance, the case in the herein investigates species *M. sculptus* and *S. seticornis* of the Majoidea (Kienbaum et al., 2017: chapter 2). These short G2 have a stout distal podomere. They have a more or less blunt tip that can have a pointed extension.

In the second case, the transformation would have involved the reduction of the formerly thinned and elongated distal podomere of the G2. This seems to be the case, for example, in Xanthidae (*Lybia tessellata* (Latreille in Milbert, 1812), pers. obs.). Here, the distal podomere has been reduced distally to the "spoon-like structure" that is still present. The same transformation has also been suggested for Calappidae (Ewers-Saucedo et al., 2016) and Bythograeidae (Mateos et al., 2012). In both groups, representatives with long and thin G2 but also species with short G2 are found. Brandis et al. (1999) regarded the apical girdle at the tip of majoid G2 as very similar to the "spoon-like structure" of the long G2 of *Potamon gedrosianum* Alcock, 1909. They reasoned a possible evolutionary correlation through the reduction of a long distal podomere and thereby indirectly homologised the apical girdle, that is present on the tip of the G2 in heterotreme and thoracotreme species (Kienbaum et al., 2017, 2018a: chapter 2, 3) with the "spoon-like structure" of long G2. However, they were also aware that this might be a premature estimation and that more and carefully examined data would be needed.

Regardless of an ongoing discussion about the monophyly of heterotremes (Jamieson et al., 1995; von Sternberg & Cumberlidge, 2001; Ahyong et al., 2007; Tsang et al., 2014; Basso et al., 2017; Yuhui et al., 2017), it is likely that at least the G2 underwent these evolutionary transformation series from long into short multiple times. In this case, even though tempting, one cannot assume that the gonopod morphology shows a strict tendency towards reduction in groups that are nested more deeply within the heterotreme tree. It seems reasonable that the proposed size relationships between G1 and G2 of a species might be less important than the actual form to refer on evolutionary scenarios.

Fig. 5.2 Drawings of the G1 and G2 (in blue and green, respectively; setae not shown). The gonopod morphology is variable and the shown gonopods are intended to exemplify their diversity within brachyuran taxa. Groups are sorted in alphabetical order. Dashed lines indicate parts that were not visible in the original figure.
"Podotremata" encircled by rose line: ***Tymolus uncifer***, after Vehof, unpublished; ***Tymidodromia dormai***, after Stephensen, 1946; ***Homola barbata*** after Becker, unpublished; ***Ranina ranina***, after Minagawa, 1994.
Heterotremata encircled by lavender line: ***Bythograea vrijenhoeki***, after Guinot & Hurtado, 2003; ***Calappula saussurei***, after Ewers-Saucedo et al., 2015; ***Cancer johngarthy***, G1, after Carvacho, 1998; ***Cancer pagurus***, G2, after George, 2004; ***Carcinus maenas***, after Spalding 1942, Beninger & Laroque, 1998; ***Trogloplax joliveti***, after Stephensen, 1946; ***Hyastenus hilgendorfi***, after Stephensen, 1946; ***Eriphia verrucosa***, after George, 2004; ***Ethusa mascarone***, after Vehof et al., 2014; ***Eucrate crenata***, after Stephensen, 1946; ***Carcinoplax purpurea***, after Stephensen, 1946; ***Stenorhynchus seticornis***, after Kienbaum et al., 2017; ***Arcania quinquespinosa***, after Stephensen, 1946; ***Matuta planipes***, after Stephensen, 1946; ***Lydia tenax***, after Stephensen, 1946; ***Enoplolambrus carenatus***, after Stephensen, 1946; ***Actumnus asper***, after Stephensen, 1946; ***Danielethus crenulatus***, after Thoma et al., 2012; ***Callinectes sapidus***, after Cronin, 1947; ***Potamon setiger***, after Brandis et al., 1999; ***Tetralia glaberrima***, after Stephensen, 1946; ***Lybia tessellata***, pers. obs..
Thoracotremata encircled by mint green line: ***Grapsus grapsus***, after Stephensen, 1946; ***Limnopilos naiyanetri***, after Kienbaum et al., 2018b; ***Uca annulipes*** (* accepted as ***Austruca annulipes***), G1, after Lautenschlager et al., 2010; ***Ocypode saratan***, G2, after Stephensen, 1946; ***Percnon gibbesi***, after Kienbaum et al., 2018a; ***Pinnotheres pisum***, after Becker et al., 2012; ***Scandarma splendidum***, after Naruse & Ng, 2007.


Cyclodorippidae
1mm
Tymolus uncifer
Dromiidae
no scale
Tumidodromia dormia
Homolidae
no scale
Homola barbata
Raninidae
5mm
5mm
Ranina ranina
Bythograeidae
2mm
5mm
Bythograea vrijenhoeki
Calappidae
1mm
600µm
Calappula saussurei
Cancridae
1cm
1mm
Cancer johngarthy, G1
Cancer pagurus, G2
Carcinidae
2mm
G2 not to scale
Carcinus maenas
Chasmocarcinidae
no scale
Trogloplax joliveti
Epialtidae
no scale
Hyastenus hilgendorfi
Eriphiidae
1mm
1mm
Eriphia verrucosa
Ethusidae
1mm
1mm
Ethusa mascarone
Euryplacidae
no scale
Eucrate crenata
Goneplacidae
no scale
Carcinoplax purpurea
Inachoididae
200 µm
100 µm
Stenorhynchus seticornis
Leucosiidae
no scale
Arcania quinquespinosa


Matutidae
no scale
Matuta planipes
Oziidae
no scale
Lydia tenax
Parthenopidae
no scale
Enoplolambrus carenatus
Pillumnidae
no scale
Actumnus asper
Platyxanthidae
no scale
Danielethus crenulatus
Portunidae
1cm
1cm
Callinectes sapidus
Potamidae
no scale
Potamon setiger
Tetraliidae
no scale
Tetralia glaberrima
Xanthidae
200µm
100µm
Lybia tessellata
Grapsidae
no scale
Grapsus grapsus
Hymenosomatidae
100µm
30µm
Limnopilos naiyanetri
Ocypodidae
1mm
no scale
Uca annulipes*,G1
Ocypode saratan,G2
Percnidae
100µm
100µm
Percnon gibbesi
Pinnotheridae
300µm
100µm
Pinnotheres pisum
Sesarmidae
1mm
1mm
Scandarma splendidum
„Podotremata"
Heterotremata
Thoracotremata
First Gonopod (G1)
Second Gonopod (G2)

THORACOTREME GONOPODS

In accordance with the herein investigated grapsoid species *P. gibbesi* (Kienbaum et al., 2018a: chapter 3) and the hymenosomatid *L. naiyanetri* (Kienbaum et al. 2018b: chapter 4), in all thoracotreme males the G2 are shorter than the G1. The G1 of thoracotremes can have a rather broad form (for example in Grapsidae: Stephensen, 1946; Fig. 5.2). If thoracotremes are the sister group to monophyletic Heterotremata (Tsang et al., 2014), the transformation of the gonopods could have evolved from the common ancestor of Eubrachyura and the podotreme sister group, possibly Cyclodorippoidea (Karasawa et al., 2011) or from the character state at the base of the Eubrachyura (Fig. 5.1). The tubulation of the G1 in thoracotremes might have resulted in a generally broader form and the short G2 would have evolved through a reduction without a preceding elongation. If the heterotremes are paraphyletic, the Potamoidea are likely to be the sister group to the monophyletic Thoracotremata (Shen et al., 2013; Basso et al., 2017; Yuhui et al., 2017). The gonopods of a potamid species have been investigated by Brandis et al. (1999). Here the G2 is longer than the G1 (Fig. 5.2). If the gonopods in both groups evolved from a common ancestor, the short G2 in thoracotremate taxa could be the result of a secondary reduction of the distal podomere. However, the long G2 in Potamidae could represent a character state of this group and the gonopods in thoracotremate taxa could still have evolved from the character state at the base of the Eubrachyura. As information on the phylogeny of the Eubrachyura are inconclusive, most hypotheses on gonopod morphology and its implications remain speculative.

In general, the male gonopods seem to be suitable to test and confirm the affiliation of species to a certain group because of the characters they share within this group. In the context of large-scale brachyuran phylogeny, male gonopod characters may still contain some useful phylogenetic signal. However, this is here predicted to be limited to certain parts of the tree only, owing to convergent evolutionary transformations in several groups. Even without a reliable brachyuran phylogeny, the latter phenomenon can be illustrated, for instance, by the co-occurrence of short *and* long G2 types *within* morphologically well-supported heterotreme taxa such as Calappidae (Ewers-Saucedo et al., 2016) or Bythograeidae (Mateos et al., 2012), which clearly underlines homoplasy in gonopod characters.

5.1.2| MUSCULATURE

Data on the musculature of gonopods are relatively scarce. This is surprising, because information on musculature provides fundamental information on possible movement and thus provides important insights for functional considerations.

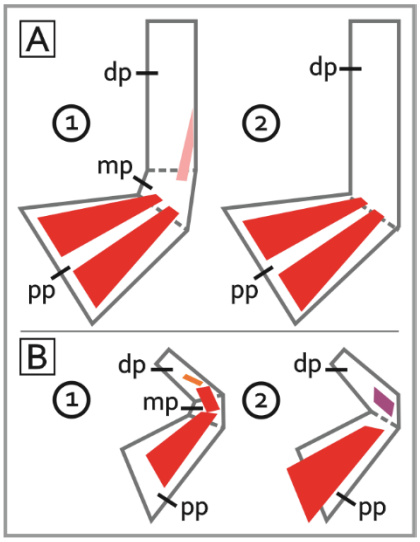


Fig. 5.3 Schematic drawing of the musculature (red) within the G1 (**A**) and the G2 (**B**) of heterotremes (**1**) and thoracotremes (**2**) based on data from Cochran (1935); Spalding (1942); Beninger et al. (1991); Brandis et al. (1999); George (2004); Sal Moyano et al. (2011); Becker et al. (2012); Kienbaum et al. (2017), (2018a): (chapter 2, 3). (**A1**) The G1 of heterotremes is tripartite. Its proximal podomere is equipped with two muscles that run to the middle podomere. The muscle that connects the middle and distal podomere (rose) is absent in Potamoidea. (**A2**) The G1 of thoracotremes is bipartite. Its proximal podomere is equipped with two muscles that run to the distal podomere. (**B1**) The G2 of heterotremes is tripartite. One muscle connects the proximal and the middle podomere. A second muscle runs between middle and distal podomere. In Majoidea a third muscle is present within the distal podomere (orange). (**B2**) The G2 of thoracotremes is bipartite. One muscle connects the proximal podomere to the pleon. In Percnidae a second muscle is present within the distal podomere (violet). This muscle may also be present in Pinnotheridae.
Abbreviations: dp – distal podomere, mp – middle podomere, pp – proximal podomere

The only data on musculature that spans between the pleon and the proximal podomere of the G1 is shown for the portunid *Callinectes sapidus* Rathbun, 1896 (Cochran, 1935). All remaining studies focus on the musculature within the gonopods.

All taxa investigated consistently possess two muscle strands within the proximal podomere (Cochran, 1935; Spalding, 1942; Brandis et al., 1999; Sal Moyano et al., 2011; Becker et al., 2012; Kienbaum et al., 2017, 2018a: chapter 2, 3) (Fig. 5.3). In podotreme and heterotreme taxa with a tripartite G1 this musculature spans to the middle podomere (Cochran, 1935; Kienbaum et al., 2017: chapter 2). In thoracotremes with a bipartite G1, the muscle strands within then proximal podomere connect to the distal podomere (Becker et al., 2012; Kienbaum et al., 2018a: chapter 3).

In podotremes and heterotremes, a third muscle connects the middle podomere and the distal podomere. Minagawa (1993) describes it in the proximal region of the distal podomere of the podotreme *Ranina ranina* (Linnaeus, 1758). Unfortunately, this is the only detailed description of gonopod morphology in podotremes, which currently hampers comparison within this paraphyletic assemblage. In heterotremes, this third muscle is present in Majoidea (Beninger et al., 1991; Kienbaum et al., 2017: chapter 2), in Portunidae (Cochran, 1935), in Cancridae and Eriphiidae (George, 2004) and in Xanthidae (*L. tessellata*; pers. obs).

Interestingly, Brandis et al. (1999) did not observe musculature within the distal podomere of the Potamidae and all thoracotremes lack this musculature in the distal podomere as well (Becker et al., 2012; Kienbaum et al., 2018a: chapter 3). Accordingly, the close phylogenetic relationship of Potamoidea and Thoracotremata that has been proposed by different molecular studies (Shen et al.,

2013; Basso et al., 2017; Yuhui et al., 2017) might be supported by this additional morphological trait. Furthermore, a tendency towards reduction of musculature within the G1 of thoracotremes, which agrees with the reduction of the podomeres, is indicated. If the musculature that connects proximal and middle podomere of podotremes, heterotremes and thoracotremes is homologised, the reduction of the podomeres in thoracotreme G1 might be the result of a fusion of middle and distal podomere. A reduction of the podomeres through the loss of the distal podomere seems unlikely, because it would necessarily have involved the elongation and tubulation of the middle podomere.

Information on the musculature of the G2 is almost absent from the literature. Again, the only depiction of musculature that connects the G2 to the pleon is shown for the portunid *C. sapidus* (Cochran, 1935). In heterotremes, the proximal podomere is equipped with one muscle that connects to the middle podomere (Fig. 5.3). As in *M. sculptus* and *S. seticornis* (Kienbaum et al., 2017: chapter 2), this musculature in the "basal region" has also been described in other majoid taxa (Beninger et al., 1991; Sal Moyano et al., 2011) and is present in the portunid *C. sapidus* (Cochran, 1935). In Majoidea, two more muscles are present. One connects the middle and distal podomere and the other, interestingly, is an additional muscle that is present only within the distal podomere (Beninger et al., 1991; Kienbaum et al., 2017: chapter 2). This third muscle might be an apomorphy of the group as it has not been described in other species so far. As shown for *Percnon gibbesi* by Kienbaum et al., (2018a) (chapter 3), in thoracotreme taxa, the musculature of the proximal podomere does not span as far as the distal podomere. Additionally, the second muscle that can be found in the G2 of this species is present in the distal podomere only. In the thoracotreme pinnotherids no musculature has been observed (Becker et al., 2012). However, observations were merely based on histological sections and an insertion of musculature from the proximal podomere to the distal podomere may have been overlooked (C. Becker, pers. com.). If the bipartite G2 of thoracotremes, is the result of a fusion of middle and distal podomere, this muscle could be homologous to the muscle that connects these podomeres in the tripartite G2 of heterotremes.

5.1.3 | CONSIDERATIONS ON SPERM TRANSPORT

In the following, with data on gonopod morphology and their musculature at hand, the ongoing discussion on sperm transport will be addressed. Observations of the copulation have been described throughout the last century (Stephensen, 1946; Hartnoll, 1969; Beninger et al., 1991; Brandis et al., 1999; Becker et al., 2012; Ewers-Saucedo et al., 2015). It is generally agreed upon that the penis extrudes the sperm by help of its musculature into the ejaculatory canal of the G1 (Ryan, 1965; Hartnoll, 1969; Minagawa, 1993). Almost all hypotheses on sperm transport address species with a short G2. Here, a piston-like pumping movement of the G2 within the proximal ejaculatory canal of the G1 is assumed (Stephensen, 1946; Cronin, 1947; Hartnoll, 1969; Bauer, 1986; Beninger

et al., 1991; Diesel, 1991; Minagawa, 1993; Becker et al., 2012). These hypotheses are based on field and laboratory observations of copulations (Hartnoll, 1969; Elner et al., 1985) and considerations addressing the gonopod morphology that are based on histological sections and data from scanning electron microscopy (Beninger et al., 1991; Minagawa, 1993; Becker et al., 2012) or μCT-scans of gonopods that provide insights about the structures within the intact gonopod (Kienbaum et al., 2017, 2018a: chapter 2, 3).

The musculature within the tripartite G1 of heterotremes probably provides a larger range of motion due to the muscle that spans from the middle podomere to the distal podomere. However, this does not seem to affect the reproductive success of the heterotreme Potamoidea, which lack this muscle and the thoracotreme species with a bipartite G1. Therefore, it is probable, that the musculature within the G1 is foremost used to position it within the female vagina. During copulation, the most important and highest range of motion can probably be attributed to the pleon that is flexed repeatedly (Elner et al., 1985).

Based on the similar orientation of the musculature within the G2, its range of motion is comparable. The additional muscle within the distal podomere of majoids led to a hypothesis on copulation by Beninger et al. (1991). It argues in favour of a piston-like movement of the G2 induced by pleon movement. He assumes the apical girdle at the tip of the G2 of majoids to act as a seal that enables the transportation of sperm and at the same time prevents its loss. Hereby, the seal breaks with each stroke through force of the muscle within the distal podomere. The structural similarities of G2 in Majoidea, also described in Kienbaum et al. (2017) (chapter 2) support this hypothesis.

Still, as shown by Kienbaum et al. (2017, 2018a) (chapter 2, 3) for *M. sculptus* and *P. gibbesi*, there is not much space for the short G2 within the ejaculatory canal and the piston-like movement is limited. The very strong cuticle at the tip of the G2 of those species is contradictory to a hypothesis of Becker et al. (2012). Here, within the distal podomere of Pinnotheridae, a swelling of the G2 might be achieved through haemolymph pressure and could thereby seal the ejaculatory canal. Even though this is an interesting assumption it needs experimental validation.

Morphological investigations associated with sperm transport in species with long G2 are almost non-existent. One major difference is the role of the G2 as the actual organ of sperm transfer that is introduced into the female system, while the broad G1 has a stabilizing role (Brandis et al., 1999; Ewers-Saucedo et al., 2015). The authors also suggested that the G2 would not perform pumping movements as do short G2. By contrast, Elner et al. (1985) describe the flexion of the pleon and an associated pumping movement of the G2 during copulation in a cancrid species with a long G2. Therefore, the pumping movement of male gonopods during copulation might not be correlated to their relative length and shape. Additionally, the transport of sperm could be realised through high

pressure induced by the very small space between the groove along the distal podomere of the G2 and the ejaculatory canal of the G1 (Brandis et al., 1999).

SETAE

Setae and denticles on the gonopods have been assigned different roles. Depending on their position and orientation, setae may mechanically assist in stabilizing the insertion of the G1 and as a possible mechanosensory modality, may additionally assist in its correct positioning (Beninger et al., 1991). This was agreed upon by Minagawa (1993), who suggested that pappose and simple setae act as contact sensors to the female abdomen and additionally, as they are positioned at the proximal opening for the G2, they could detect its insertion into the G1. The presence of pappose setae at the proximal opening for the G2 and penis has also been shown for the Majoidea *M. sculptus* and *S. seticornis* (Kienbaum et al., 2017: chapter 2) and the thoracotreme *P. gibbesi* (Kienbaum et al., 2018a: chapter 3). This special position leads to another possible role of pappose setae as passive filter/grooming structures that prevent particles from entering the ejaculatory canal and finally the female system.

Chemosensory function as well as removal of competitive sperm from the female seminal receptacle by setae at the G1 tip has also been hypothesised by Beninger et al. (1991).

Denticles at the tip and within the ejaculatory canal of the G1 of the majoid *Inachus phalangium* (Fabricius, 1775) have been assumed to rupture spermatophores during copulation (Rorandelli et al., 2008). This was opposed by Sal Moyano et al. (2011) based on the location of this type of setae. Kienbaum et al. (2017) (chapter 2) agree that such a rupture of spermatophores is unlikely, as intact spermatophores were observed in the seminal receptacle of *S. seticornis,* a majoid species that also has denticles at the G1 tip (Antunes et al., 2016, 2018). Sal Moyano et al. (2011) alternatively suggested that these denticles support the anchoring of the G1 tip within the female system during copulation. A rough cuticle surface, such as the one created by the denticles at the G1 tip, could actually support this role.

GONOPOD TEGUMENTAL GLANDS

The tegumental glands can be distributed across the entire body but can also be restricted to a specific location (for a thorough review on tegumental glands see Talbot & Dehmers, 1993). Within the gonopods of male brachyurans they are termed gonopod tegumental glands. In all species investigated, they were positioned around the ejaculatory canal within the G1 and communicate with its lumen via ducts which pass through large pores (Beninger et al., 1995; Beninger & Larocque, 1998; Brandis et al., 1999; Becker et al., 2012). It is generally agreed that the sperm is transferred through the ejaculatory canal of the G1 and that all ducts of the tegumental glands lead into this ejaculatory

canal. The secretory cells of the tegumental glands can produce two different types of secretions, namely acid mucopolysaccharides (AMPS) and neutral mucopolysaccharides (NMPS) (Talbot & Demers, 1993; Beninger et al., 1995; Beninger & Larocque, 1998). Different functions were attributed to these secretes, supported by the investigation of Beninger et al. (1995) and Beninger & Larocque (1998). Both studies suggested a role as accessory sex glands, a hypothesis that found support by consecutive studies (Brandis et al., 1999; Becker et al., 2012). It is probable, that during copulation, the gonopod tegumental glands facilitate the transport of sperm by secreting the NMPS into the ejaculatory canal. Their position around the proximal opening of the ejaculatory canal conforms to the site of insertion of the G2. Assuming that the G2 plays an assisting role during sperm transport, a lubricant that reduces the mechanical wear within the ejaculatory canal would be supportive.
The AMPS is more viscous and might be antimicrobial and constitute layers of sealants within the female seminal receptacle when transferred after the sperm (Johnson, 1980; Jensen et al., 1996; Beninger & Larocque, 1998). This is in agreement with the findings of Antunes et al. (2016) who found separate sperm packets delimited by acidophilic secretions in the seminal receptacle of female *S. seticornis*. Both, older and newer sperm packets contained NMPS but the ratio of AMPS varied and was low in older sperm packets while it was high in the fresher one. Additionally, a layer of AMPS separated the newest sperm packet from the others. Interestingly, when Kienbaum et al. (2017) (chapter 2) investigated the sperm mass within the seminal receptacle of *S. seticornis*, no separate sperm packets or any apparent sperm layering were found. Here, the seminal receptacle was filled with free spermatozoa only. This is probably due to the time elapsed since mating and the disintegration or removal of AMPS secretions with time.

Generally, even though some functional aspects, as for example the small space for the G2 within the ejaculatory canal or the secretion of the gonopod tegumental glands, remain unresolved for now, the prevailing hypotheses associated with sperm transport in species with short G2 are mostly supported by the data on gonopod morphology from Kienbaum et al. (2017, 2018a) (chapter 2, 3).

5.2 | THE REPRODUCTIVE SYSTEM OF FEMALE BRACHYURA

When Diesel (1991) proposed his theory about the dorsal and ventral type seminal receptacle, most of the species investigated had been commercially important Majoidea (Hartnoll, 1968; Beninger et al., 1988; Diesel, 1989, 1991) and some Portunoidea (Spalding, 1942; Ryan, 1965; Hartnoll, 1968; Johnson, 1980). Thereafter, even more studies of the majoid reproductive system followed (Lanteigne et al., 1996; Sainte-Marie & Sainte-Marie, 1998; Rotllant et al., 2007; Sal Moyano et al., 2010; González-Pisani et al., 2011; Antunes et al., 2016; Kienbaum et al., 2017: chapter 2). The Majoidea are large groups of approximately 800 species (De Grave et al., 2009), representing about 10% of all brachyurans (Ng et al., 2008). They have been argued to be an early diverging lineage within heterotremes (Spears et al., 1992; Jamieson et al., 1995; Porter et al., 2005). However, the strict ventral/dorsal division of the seminal receptacle might be applicable to some majoids, but has been shown to be different in *M. sculptus* and *S. seticornis* in Kienbaum et al. (2017) (chapter 2). Additionally, as more exceptions from this "dorsal/ventral division" emerge (Calappidae: Ewers-Saucedo et al., 2015; Cancridae: Ohrensanz et al., 1995; Jensen et al., 1996; Oh & Hankin, 2004; George, 2004; Pardo et al., 2013; Dorippidae: Hayer et al., 2016; Vehof et al., 2017, 2018; Leucosiidae: Hayer et al., 2014, 2017; Ocypodidae: Lautenschlager et al., 2010), it seems most likely that the majoid seminal receptacle is not the blueprint for every other eubrachyuran group, but "simply" a seminal receptacle with a large dorsal secretory area. Unfortunately, the convenient hypothesis of Diesel (1991), who proposed the existence of a "storage" and a "fertilisation chamber" within the seminal receptalce of majoids, continues to influence the interpretation of these structures in more recent studies.

Fortunately, more data on representatives of different heterotreme groups that probably belong to very early diverging lineages (Majoidea: Lanteigne et al., 1996; Sainte-Marie & Sainte-Marie, 1998; Antunes et al., 2016, Kienbaum et al., 2017: chapter 2; Dorippidae: Hayer et al., 2016; Vehof et al., 2017, 2018; Ethusidae: Hayer et al., 2016) but also to groups deeply nested within the eubrachyuran tree (Cancridae: Pardo et al., 2013; Jensen et al., 1996; Menippidae: de Souza et al., 2017; Portunidae: Zara et al., 2014; Geryonidae: Pardo et al., 2017) emerge. While the character states of the heterotreme reproductive systems show specific variability, there is an overall tendency towards more morphological uniformity within thoracotremes. Still, it has now become clear that there is a much higher diversity in female reproductive systems than previously assumed (Fig. 5.4, 5.5). With these new data, it is possible to focus in more detail on character states that allow comparison between the eubrachyuran groups (Fig. 5.4).

5.2.1 | COMPARISON OF CHARACTER STATES IN EUBRACHYURAN GROUPS

Data on the reproductive system is available for the following taxa. For an overview they are sorted in alphabetical order after Ng et al. (2008). In contrast to Ng et al. (2008) Hymenosomatoidea are considered as a thoracotreme group (Kienbaum et al., 2018b: chapter 4). Species names and references are listed in table 5.1.

Heterotremata:

- Calappoidea – Calappidae
- Cancroidea – Cancridae
- Dorippoidea – Dorippidae, Ethusidae
- Eriphioidea – Eriphiidae, Menippidae, Platyxanthidae
- Leucosioidea – Leicosiidae
- Majoidea – Epialtidae, Inachidae, Inachoididae, Majidae, Oregoniidae
- Portunoidea – Carcinidae, Geryonidae, Portunidae
- Xanthoidea – Xanthidae

Thoracotremata:

- Cryptochiroidea - Cryptochiridae
- Grapsoidea – Gecarcinidae, Grapsidae, Percnidae, Varunidae
- Hymenosomatoidea - Hymenosomatidae
- Pinnotheroidea - Pinnotheridae
- Ocypodoidea - Ocypodidae

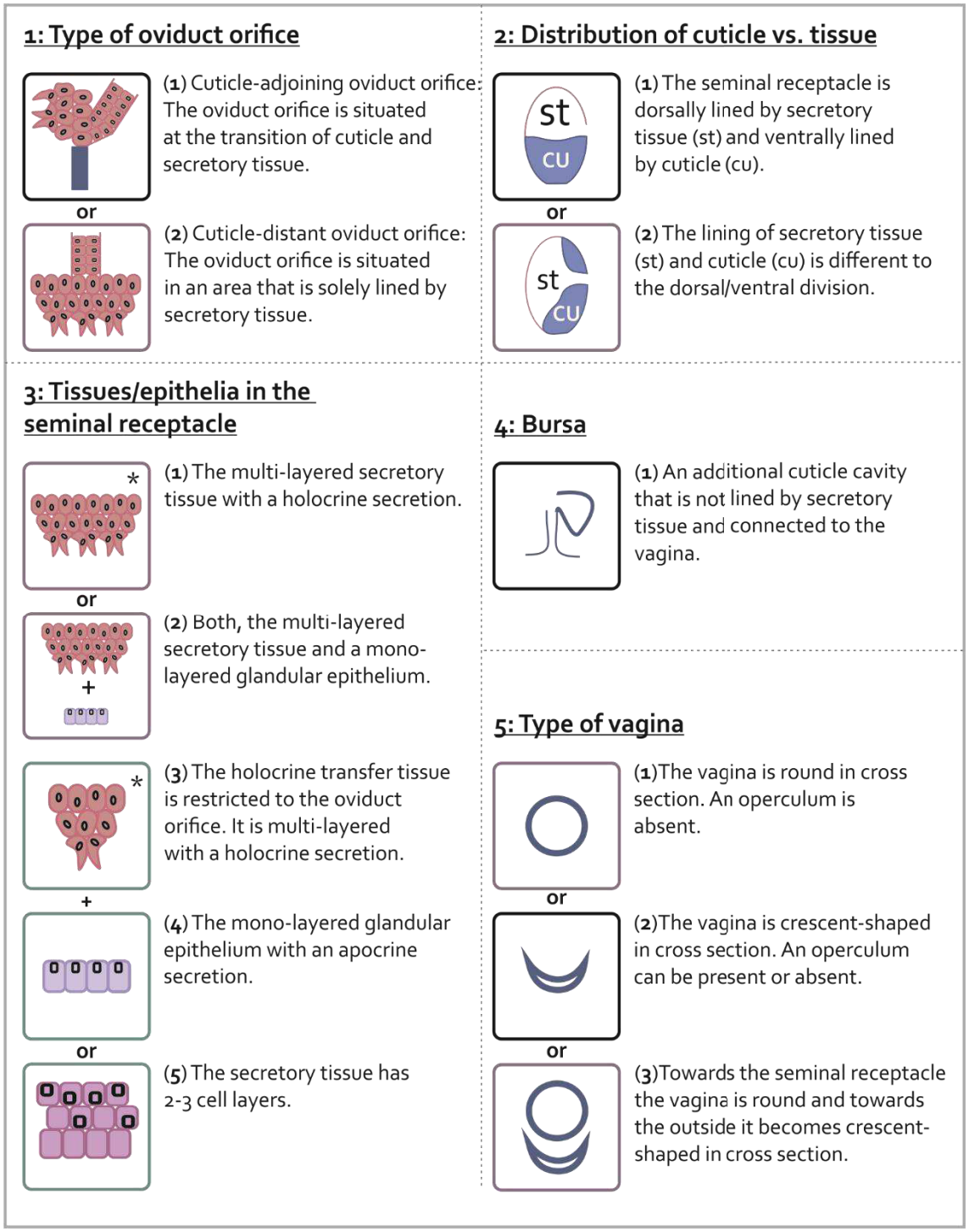


Fig. 5.4 Description of the character complexes of the female reproductive system. (**1**) Types of oviduct orifices. (**2**) Different relations of cuticle and tissue within the seminal receptacle. (**3**) Different types of tissue within the seminal receptacle. 3.3 is either present with 3.4 or with 3.5 (**4**) A bursa is present. (**5**) The shape of the vagina in cross section. Of the described character states, only one character state can occur within a species at a time (e.g. 5.1 *or* 5.2 *or* 5.3). Heterotreme character states: encircled by lavender line; thoracotreme character states: encircled by mint green line; character states present in heterotremes and thoracotremes: encircled in black line. * 3.1 and 3.3 can be homologised but are mentioned separately due to differences in the distribution within the seminal receptacle of heterotremes and thoracotremes.

Table 5.1 Character distribution of the complexes 1 – 5 described in **fig. 5.4** in eubrachyuran females. Groups are sorted after Ng et al. (2008) with the exception of Hymenosomatoidea, that are considered thoracotreme (Kienbaum et al., 2018b). **?** = no information available; **-** = absent; **°** = the secretory tissue is lined by cuticle; **°°** = only the mono-layered glandular epithelium is described; ***** = the secretory tissue is absent; ****** = two bursae; ******* = the oviduct enters a separate cuticle duct and not the seminal receptacle

group	species	reference	1	2	3	4	5
Calappidae	*Calappa saussurei*	Ewers-Saucedo et al., 2015	1.1	2.1	3.1	-	5.3
	Calappa pelii	Ewers-Saucedo et al., 2015	1.1	2.1	3.1	-	5.3
Cancridae	*Metacarcinus edwardsii*	Pardo et al., 2013	1.1	2.2	3.1	-	5.1
	Metacarcinus magister	Jensen et al., 1996; Oh & Hankin 2004	1.1	2.2	3.1	4.1	5.1
	Metacarcinus gracilis	Ohrensanz et al., 1995	1.1	2.2	3.1	-	5.1
	Cancer pagurus	George 2004 (Diploma thesis in german)	1.1	2.1	3.1	-	5.1
Dorippidae	*Dorippe sinica*	Hayer et al., 2016; Vehof et al. 2017	1.1	2.2	3.1°	4.1	5.2
	Dorippe quadridens	Vehof et al., 2017	1.1	2.2	3.1°	4.1	5.2
	Medorippe lanata	Vehof et al., 2017	1.1	2.2	3.1	4.1	5.2
	Paradorippe granulata	Vehof et al., 2018	-	*	-	4.1 **	-
Ethusidae	*Ethusa mascarone*	Hayer et al., 2016; pers. comm. Vehof	1.1	2.1	3.1	-	5.2
Eriphiidae	*Eriphia verrucosa*	George 2004 (Diploma thesis in german)	1.1	2.1	3.2	-	5.3
	Eriphia gonagra	de Souza et al., 2016	1.1	2.1	3.1	-	5.1
Menippidae	*Menippe nodifrons*	de Souza et al., 2016	1.1	2.1	3.2°°	-	5.1
Platyxanthidae	*Danielethus crenulatus*	Farias et al., 2017	1.2	2.1	3.2	-	5.2
Leucosiidae	*Ilias nucleus*	Hayer et al., 2017	1.1	2.2	3.1	-	5.2
	Persephona mediterranea	Hayer et al., 2017	1.1	2.2	3.1	-	5.2
	Ebalia tumefacta	Hayer et al., 2014	1.1	2.2	3.1	-	5.2
Oregoniidae	*Chionoecetes opilio*	Beninger et al., 1988, 1991, 1993; Lanteigne et al., 1996; Sainte-Marie & Sainte-Marie, 1998; Sainte-Marie et al., 2000; Benhalima & Moryiasu, 2001;	1.1	2.1	3.1	-	5.2
	Hyas araneus	Hartnoll 1968	1.1	2.1	3.1	-	5.2
	Hyas coarctatus	Hartnoll 1968; Lanteigne et al., 1996	1.1	2.1	3.1	-	5.2
Inachidae	*Inachus phalangium*	Diesel 1989, 1991; Rorandelli 2008	1.1	2.1	3.1	-	5.2
Inachoididae	*Stenorhynchus seticornis*	Antunes et al., 2016; Kienbaum et al., 2017	1.1	2.1	3.1	-	5.2
	Leurocyclus tuberculosus	Gonzalez-Pisani et al., 2011	1.1	2.1	3.1	-	5.2
Majidae	*Maja brachydactyla*	Rottland et al., 2007	1.1	2.1	3.1	-	5.2

	Mithraculus sculptus	Kienbaum et al. 2017	1.1	2.2	3.1	-	5.2
Epialtidae	*Libinia spinosa*	Sal Moyano et al., 2010, 2011; Gonzales-Pisani et al., 2011	1.1	2.1	3.1	-	5.2
Carcinidae	*Carcinus maenas*	Spalding 1942, Hartnoll 1968	1.2	2.1	3.1	-	?
Portunidae	*Araneus cribarius*	Zara et al., 2014	1.2	2.1	3.2	-	?
	Portunus sanguinolentus	Ryan 1965 (Thesis)	?	2.1	3.1	-	5.1
	Callinectes sapidus	Hartnoll 1968; Johnson 1980	1.2	2.1	3.2	-	?
	Callinectes bocourti	de Souza et al., 2016	1.2	2.1	3.1	-	5.1
Geryonidae	*Chaecon chilensis*	Pardo et al., 2017	1.2	2.1	3.2	-	5.1
Xanthidae	*Neopanope sayi*	Swartz 1978	1.2	?	3.1	-	5.2
	Lybia tessellata	pers. obs.	1.2	2.2	3.1	-	5.2
Cryptochiridae	*Fungicola syzygia*	Vehof et al., 2015	1.1	2.1	3.3 + 3.4	-	5.2
	Opecarcinus cathyae	Vehof et al., 2015	1.1	2.1	3.3 + 3.4	-	5.2
	Pseudocryptochirus viridis	Vehof et al., 2015	1.1	2.1	3.3 + 3.4	-	5.2
Gecarcinidae	*Cardisoma guanhumi*	de Souza et al., 2013, 2016	1.1	2.1	3.3 + 3.4	-	5.2
Grapsidae	*Goniopsis cruentata*	de Souza & Silva 2009; de Souza et al., 2016	1.1	2.1	3.3 + 3.4	-	5.2
Varunidae	*Eriocheir sinensis*	Lee & Yamazaki, 1990	1.1	2.1	3.3 + 3.4	-	5.2
Percnidae	*Percnon gibbesi*	Kienbaum et al., 2018a	1.1 ***	2.1	3.3 + 3.4	4.1	5.2
Hymenosomatidae	*Limnopilos naiyanetri*	Klaus et al., 2014; Kienbaum et al., 2018b	1.1 ***	2.1	3.3 + 3.4	4.1	5.2
Pinnotheridae	*Calyptraeotheres garthi*	Ocampo et al., 2018	1.1	2.1	3.3 + 3.4	-	5.2
	Pinnotheres pisum	Becker et al., 2011	1.1	2.1	3.3 + 3.4	-	5.2
	Pinnotheres pectunculi	Becker et al., 2011	1.1	2.1	3.3 + 3.4	-	5.2
	Nepinnotheres pinnotheres	Becker et al., 2011	1.1	2.1	3.3 + 3.4	-	5.2
Ocypodidae	*Ucides cordatus*	Sant`Anna et al., 2007; Castilho-Westphal et al., 2013; de Souza et al., 2016	1.1	2.1	3.3 + 3.5	-	5.2
	Ocypode quadrata	Lopez-Greco et al., 2009	1.1	2.1	3.3 + 3.5	-	5.2
	Uca ecuadoriensis	Lautenschlager et al., 2010	1.1	2.1	3.3 + 3.5	-	5.2
	Uca tangeri	Lautenschlager et al., 2010	1.1	2.1	3.3 + 3.4 + 3.5	-	5.2
	Uca c.f. forcipata	Lautenschlager et al., 2010	1.1	2.1	3.3 + 3.4	-	5.2
	Uca maracoani	de Souza et al., 2016	1.1	2.1	3.3 + 3.4	-	5.2

OVARY AND OVIDUCT

Two ovarian strands run dorso-laterally along both sides of the body and are connected by a bridge ventrally to the heart, resulting in an H-shaped-system. The extent and dimensions of the ovarian lobes is correlated with the reproductive cycle and ranges from small and thin to seemingly filling the entire body (de Souza & Silva, 2009). Regardless of the stage in the reproductive cycle, in most heterotremes investigated, the ovarian lobes are restricted to the carapace, while their extension into the pleon has been reported only for few representatives (Portunidae: *Portunus sanguinolentus* (Herbst, 1783) - only the right posterior lobe (Ryan, 1965), Cancridae: *Metacarcinus magister* (Dana, 1852) - only the right posterior lobe (Jensen et al., 1996; Oh & Hankin, 2004), Xanthidae: *L. tessellata*- only the left posterior lobe, (pers. obs.)). Interestingly, in some majoids, including the herein investigated *S. seticornis*, the extension of both posterior lobes into the pleon seems to be linked with their fusion (Rotllant et al., 2007; González-Pisani et al., 2011; Antunes et al., 2016; Kienbaum et al., 2017: chapter 2). In contrast to this, the ovaries of several thoracotreme species extend into the pleon but a posterior fusion of the lobes as in majoids does not occur (Becker et al., 2011; de Souza et al., 2013; Vehof et al., 2016; Ocampo et al., 2018).

The ovaries are not only simple sacs but are made of convoluted ducts of a mono-layered epithelium formed by follicle cells (Becker et al., 2011; Becker & Scholtz, 2017; Kienbaum et al., 2017: chapter 2). Some of these ducts are filled with oogonia and early previtellogenic oocytes. They represent the germinative zones of the ovary.

In some eubrachyuran groups, such as, the heterotreme Platyxanthidae (Farias et al., 2017), the Ethusidae (Hayer et al., 2016) and the Portunidae (Johnson, 1980) or the thoracotreme Pinnotheridae (Becker et al., 2011), Grapsidae (de Souza & Silva, 2009) and Percnidae (Kienbaum et al., 2018a: chapter 3), germinative zones are only present in the central area of the ovary, whereas in other species like *M. sculptus* and *S. seticornis* of the Majoidea (Kienbaum et al., 2017: chapter 2), Cryptochiridae (Vehof et al., 2016) and Ocypodidae (Castilho-Westphal et al., 2013) the germinative zones stretch throughout the ovaries in a more complex organisation and intermingle with developing and mature oocytes. There seems to be no apparent pattern that would allow drawing conclusions about an evolutionary polarity regarding the complexity of the ovaries. Additionally, ovaries are subject to strong morphological fluctuations due to seasonal changes in the female reproductive cycle, which makes them difficult to interpret. Still, the position of the oocytes within the ovary correlates with the developmental stage. The further advanced this stage is, the farther away from the germinative zones the oocytes are positioned.

Ovary and oviduct are continuous structures. Accordingly, it can be assumed that the oviduct is not a separable part within the female reproductive system but represents an extension of the ovaries (Hard, 1942; Spalding, 1942; Hartnoll, 1968; Becker et al., 2011; Kienbaum et al., 2017: chapter 2).

The oviduct is a thin duct that, depending on the position of the ovaries, is either very short – as, for example, in *Metacarcinus edwardsii* (Bell, 1835) (Pardo et al., 2013) – or a long duct running along the length of the seminal receptacle – as, for example, in *Percnon gibbesi* (Kienbaum et al., 2018a: chapter 3). The oviduct is composed of few (mostly two) cell layers. The variability of described cell shapes from squamous, cuboidal or columnar epithelium might be due to the reproductive cycle and therewith associated changes (Sainte-Marie & Sainte-Marie, 1998; Becker et al., 2011; Pardo et al., 2013). It is embedded in connective tissue and can additionally be surrounded by musculature, as is the case in some representatives of Oregoniidae (*Chionoecetes opilio* (O. Fabricius, 1788), Saint-Marie & Sainte-Marie, 1998), Cancridae (*M. edwardsii,* Pardo et al., 2013) and Leucosiidae (*Ilia nucleus* (Linnaeus, 1758), *Persephona mediterranea* (Herbst, 1794), *Ebalia tumefacta* (Montagu, 1808), Hayer et al., 2014, 2017) as well as in some thoracotreme Ocypodidae (Lautenschlager et al., 2010). In most cases, the oviduct has no apparent lumen and ends as a dead end in the multi-layered secretory tissue of the seminal receptacle. An open passage is only formed during ovulation (Hartnoll, 1968; Lee & Yamazaki, 1990; Sainte-Marie & Sainte-Marie, 1998). Secretions are present within the transition zone of the oviduct and the multi-layered secretory tissue of the seminal receptacle in few heterotreme species (Beninger et al., 1993; Vehof et al., 2017, 2018; *L. tessellata*, pers. obs.) and absent in thoracotremes. The holocrine transfer tissue that is located directly at the oviduct orifice in all thoracotremes and also produces secretions will be discussed below.

THE OVIDUCT ORIFICE AND THE PROPOSAL OF A NEW CLASSIFICATION (CHARACTER STATES 1.1-1.2)

Diesel (1991) distinguished two different types of oviduct orifice positions. The ventral position (= ventral-type seminal receptacle) was characterised as close to the vagina, while the dorsal oviduct orifice enters the seminal receptacle dorsally (=dorsal-type seminal receptacle) in some distance to the ventral vagina. With the knowledge of the last decades' studies, it must be pointed out that this classification misses one additional aspect. The "ventral" oviduct orifice is not only positioned close to the vagina but also always connected to a multi-layered secretory tissue directly at the transition to the cuticle lining as it is also the case in the investigated majoids *M. sculptus* and *S. seticornis* (Kienbaum et al., 2017: chapter 2). Therefore, its position in the seminal receptacle depends on the ratio of cuticle lining vs. secretory tissue. Additionally, in most eubrachyuran species, the oviduct orifice is located on the ventro-medial side of the seminal receptacle and not as sometimes illustrated on the ventro-lateral side (McLay & Becker 2015; Hayer et al., 2016). By contrast, the "dorsal" oviduct orifice is positioned distant to the vagina but also widely surrounded by the multi-layered secretory tissue without adjacent cuticle lining (Zara et al., 2014; de Souza et al., 2016; Pardo et al., 2017; Farias et al., 2017). Additionally, a different amount of sperm mass within seminal receptacles of the same species could result in a relatively variable oviduct orifice location due to its

high degree of flexibility. Thus, it needs to be stressed that both categories are not characterised by the location of the oviduct orifice but by the distribution of tissues in its proximity. Therefore, a new classification into a (1) *cuticle-adjoining oviduct orifice* and a (2) *cuticle-distant oviduct orifice* is proposed to express more adequately the actual structural differences. These new categories additionally avoid misunderstandings about the location of the oviduct orifice that might arise because of a high degree of cuticle-lining within the seminal receptacle. For example, McLay & López-Greco (2011) focused on hypotheses about reproductive success and by doing so, added the intermediate type for oviduct connections "somewhere in between these extremes" (of "ventral" and "dorsal-type"). This might be informative for theories on sperm competition or reproductive success. However, from a morphological point of view this third classification is of no avail, since all "intermediate" types correspond to the *cuticle-adjoining oviduct orifice*.

In heterotreme crabs, seminal receptacles with either a *cuticle-adjoining oviduct orifice* or a *cuticle-distant oviduct orifice* are found. The latter type is present in all Carcinidae (Spalding, 1942), Portunidae (Ryan, 1965; Johnson, 1980; Zara et al., 2014; de Souza et al., 2017), Geryonidae (Pardo et al., 2017), Platyxanthidae (Farias et al., 2017) and Xanthidae (Swartz 1978; pers. obs.) studied so far. In all thoracotremes, the oviduct orifice is situated in close proximity to cuticle, without any known example of a *cuticle-distant oviduct orifice*.

SEMINAL RECEPTACLE AND BURSA
(CHARACTER STATES 2.1-2.2; 3.1-3.5; 4.1)

The seminal receptacle of eubrachyuran crabs is the most intriguing part of the reproductive system. It enables females to store sperm and use it, independently from an encounter with a male, for consecutive fertilisations of eggs. Due to its specific tissue properties, females can keep the stored sperm through moults, which uncouples the fertilisation process from copulation.

The seminal receptacle has a straight, sac-like shape within the body cavity, one area being lined by a secretory tissue and the second area lined by cuticle that is continuous with the vagina. In some groups, the two different tissues are not horizontally orientated but longitudinally. For instance, the seminal receptacle of the Calappidae has a lateral–medial arrangement of the two areas (Ewers-Saucedo et al., 2015), as is the case in representatives of Dorippidae (Hayer et al., 2016; Vehof et al., 2017) and Leucosiidae (Hayer et al., 2015, 2017). The cancrid *Metacarcinus magister* (Jensen et al., 1996) and the xanthid *Lybia tessellata* (pers. obs.) also possess longitudinally orientated areas within the seminal receptacle. In contrast to the diverse heterotremes, all thoracotreme species investigated have a dorsal glandular epithelium and a ventral cuticle lining, the exception being only few representatives of ocypodids that have the longitudinal type of division (Lautenschlager et al., 2010). The occurrence in both patterns in heterotremes as well as in thoracotremes illustrates again convergent transformations, irrespective of the underlying phylogeny.

Of the two different types of lining present in the heterotreme seminal receptacle the secretory tissue exhibits striking tissue architecture. As it is the case in the investigated majoid species *M. sculptus* and *S. seticornis*, secretions are produced by an area of a multi-layered secretory tissue (Kienbaum et al., 2017: chapter 2). Its cell layers can be divided into three different strata. The outermost anchoring stratum is composed of cells of the secretory tissue that intermingle with the surrounding connective tissue. In the middle stratum, these cells proliferate and then degenerate in the innermost stratum in form of holocrine secretion into the seminal receptacle lumen. The multi-layered secretory tissue merges into a columnar epithelium that is lined by cuticle. At the transition, cuticle folds are often found to protrude into the seminal receptacle lumen (Lanteigne et al., 1996; Becker et al., 2011; González-Pisani et al., 2011; de Souza et al., 2013; Kienbaum et al., 2017: chapter 2).

Interestingly, another type of mono-layered glandular epithelium within the seminal receptacle of Portunoidea, the "modified dorsal epithelium" has been described at the transition zone between the ventral cuticle area and the dorsal secretory area (Johnson, 1980; Zara et al., 2014; Pardo et al., 2017). It has large, basally multi-nucleated, columnar cells with microvilli that release their secretions into the seminal receptacle lumen. In some representatives of Dorippidae (Vehof et al., 2017), Menippidae (de Souza et al., 2017) and Eriphiidae (George, 2004), similar cells have been described as "atypical columnar cells". These cell types have also been found in Platyxanthidae (Farias et al., 2017) but are correlated to the reproductive cycle and depend on the amount of stored sperm. Here, in contrast to the "modified dorsal epithelium" and the "atypical columnar cells" of the other taxa, they are not situated at the transition zone of cuticle and multi-layered secretory tissue but line the secretory tissue itself, in close proximity to the oviduct orifice (Farias et al., 2017).

Both, the "modified dorsal epithelium" and the "atypical columnar cells" show some similarity with descriptions of the mono-layered glandular epithelium in the seminal receptacle of thoracotremes (Becker et al., 2011; Vehof et al., 2016). However, this is probably due to convergence, because its position and appearance within the seminal receptacle does not coincide with the position of the thoracotreme mono-layered glandular epithelium. This also supports the suggestion that the binary subdivision of the seminal receptacle into a dorsal glandular area and a ventral cuticle-lined region might be too simplified.

The seminal receptacles of all thoracotremes feature two different types of secretory tissues. The mono-layered glandular epithelium lines the dorsal area of thoracotreme seminal receptacles. It has been described as being composed of cells that can project into the seminal receptacle lumen, as for example in Pinnotheridae (Becker et al., 2011; Ocampo et al., 2018) or that form a smooth surface, as in the herein investigated percnid *P. gibbesi* (Kienbaum et al., 2018a: chapter 3) and hymenosomatid *L. naiyanetri* (Kienbaum et al., 2018b: chapter 4). All cells possess large, basally situated nuclei that might appear as several nuclei in histological section due to strong folding (Becker et al., 2011). In

contrast to the heterotremes studied, it is this mono-layered glandular epithelium that lines the main secretory area of the seminal receptacle and can be interpreted as an apomorphic feature of the female reproductive system in thoracotremes. In some taxa, the mono-layered glandular epithelium has been reported to be equipped with microvilli (Ocypodidae - Lautenschlager et al., 2010; Pinnotheridae - Becker et al., 2011; Cryptochiridae - Vehof et al., 2016). Unfortunately, most histological methods provide resolutions that do not cover the size of microvilli, which currently impedes evaluation of this characteristic across all thoracotreme taxa.

The second type of secretory tissue is restricted to the oviduct orifice. The holocrine transfer tissue (sensu Becker et al., 2011) is multi-layered and consists of densely packed, small cells with oval nuclei. It sheds a holocrine secretion into the seminal receptacle lumen. Lee & Yamazaki (1990) studied the structural changes of the holocrine transfer tissue (coined "valve-like" tissue in their study) during oviposition in *Eriocheir sinensis* H. Milne Edwards, 1853. They proposed that the oviduct structure and its tissue properties at the orifice present differences when compared to heterotremes, a conclusion that turned out to be misguided. On the contrary, due to its position and properties, the holocrine transfer tissue can be homologised with the multi-layered secretory tissue that lines the seminal receptacle of heterotreme species (McLay & Becker, 2015; Kienbaum et al., 2018a, 2018b: chapter 3, 4). Both tissues differ in the extension within the seminal receptacle.

Interestingly the seminal receptacle of some thoracotreme Ocypodidae is not lined by the mono-layered glandular epithelium but a different type of secretory tissue (Sant'Anna et al., 2007; López-Greco et al., 2009; Lautenschlager et al., 2010; Castilho-Westphal et al., 2013). Here, the secretions are produced by a tissue of two to three cell layer-thickness. It has different properties to the multi-layered secretory tissue of heterotremes but does not represent the thoracotreme mono-layered glandular epithelium either.

Very few of the investigated eubrachyuran species possess an accessory cuticle-lined cavity for sperm storage, the bursa, as part of the female reproductive system. To date, it has only been found in the cancrid species *M. magister* (Jensen et al., 1996), all Dorippidae hitherto studied (Vehof et al., 2017, 2018), the percnid species *P. gibbesi* (Kienbaum et al., 2018a: chapter 3) and the hymenosomatid species *L. naiyanetri* (Klaus et al., 2014; Kienbaum et al., 2018b: chapter 4).

Bursae have been discussed in the context of sperm competition in *M. magister* by Jensen & Bentzen (2012). In this species, a sperm plug produced by the primary male (the first male to mate per season), prevents the sperm of consecutive copulations from entering the seminal receptacle but does not cover the genital opening and bursa. This results in sperm from secondary males to enter the bursa only, which is then lost during moult and not used for fertilisation. Therefore, only primary males from consecutive years are involved in sperm competition. Since not all of the sperm is used for fertilisation there is often more than one sperm packet in the seminal receptacle of multiparous

females, each from a primary male per season. Fertilisation experiments also indicate a last (primary) male precedence in *M. magister* (Jensen & Bentzen, 2012). However, these insights into sperm competition cannot be extrapolated to all eubrachyurans with a bursa. In fact, it may hold only for *M. magister* since the opening of its bursa is farther away from the seminal receptacle and the sperm plug can prevent consecutive insemination in the latter. In all other species possessing a bursa, namely the Dorippidae (Vehof et al., 2017), and the herein studied *P. gibbesi* and *L. naiyanetri* (Kienbaum et al., 2018a, 2018b: chapter 3, 4), its opening is very close to the oviduct orifice and the seminal receptacle. An effective separation of the bursa from the seminal receptacle via a plug seems unlikely. The reproductive system of *Paradorippe granulata* (De Haan, 1841) consists of two bursae per side and the oviduct orifice located between them (Vehof et al., 2018). It remains unclear if one of these bursae can be homologised with the bursae or the seminal receptacle of other species. However, since the seminal receptacle is missing in *P. granulata*, the bursae content must be involved in the fertilisation process. Thus, with the exception of *M. magister*, it can be assumed, that the bursa is part of the general sperm storage structures in eubrachyurans and in this context might take part in sperm competition. Another hypothesis concerning the involvement of the bursa in brachyuran reproduction is that of cryptic female choice (Klaus et al., 2014). There, it has been proposed that the female actively controls whether sperm enters the seminal receptacle or the bursa. Based on structural considerations of the reproductive system of *L. naiyanetri*, this has been questioned by Kienbaum et al. (2018b) (chapter 4). Cryptic female choice does not generally seem to involve the bursa, especially if sperm from the bursa is used for fertilisation.

VAGINA
(CHARACTER STATES 5.1-5.3)

The vagina is completely cuticle-lined in all eubrachyuran species. Its diversity has been thoroughly investigated by Hartnoll (1968). This resulted in a classification of two different types of vaginae. The "simple" type is round in cross section. It can be found in Cancridae (Ohrensanz et al., 1995; Jensen et al., 1996; George, 2004; Pardo et al., 2013), all representatives of Portunoidea (Spalding, 1942; Ryan, 1965; Hartnoll, 1968; Zara et al., 2014; de Souza et al., 2017; Pardo et al., 2017) and some Eriphiidae and Menippidae (de Souza et al., 2017). Most of the remaining heterotremes and all thoracotreme species have a vagina with a crescent shape in cross section (Guinot et al., 2013). A vagina that consists of both, an outwards crescent shape and the round shape towards the seminal receptacle has been found in the two investigated calappids *Calappa saussurei* Rathbun, 1898 and *Calappa pelii* Herklots, 1851 (Ewers-Saucedo et al., 2015) as well as the eriphiid *Eriphia verrucosa* (Forskål, 1775) (George 2004).

One can argue that the continuance of the vagina cuticle with the sternite and the invagination of one side of the vagina wall inevitably resulted in the appearance of a "cover" from the outside. The

additional increase of cuticle thickness in this "cover" area and its decrease at its "hinge" led to a sometimes prominent cuticle structure that has been termed operculum. The operculum is a structure that closes the vagina towards the outside (Hartnoll, 1968). It is absent in the round vagina and can (but need not) be present in the crescent-shaped vagina (Hartnoll, 1968; Guinot et al., 2013). However, the subtle differences of the operculum – or "vulva cover" (McLay & Sal Moyano, 2016) – make this concept difficult to grasp and resulted in different interpretations throughout the literature (Hartnoll, 1968; Thompson & McLay, 2005; Guinot et al., 2013; McLay & Sal Moyano, 2016). Regardless of which term is used, it remains the continuance of the inner vagina wall.

5.2.2 | CONSIDERATIONS ON THE EVOLUTION OF THE REPRODUCTIVE SYSTEMS

A direct connection of the ovaries and the sperm storage location is a unique character of all Eubrachyura (Guinot, 1977; Guinot & Quenette, 2005; but see Vehof et al., 2018) but is absent in other decapod species including podotreme crabs (Bauer, 1986; Minagawa, 1993; Guinot & Quenette, 2005; Becker & Scholtz, 2017). Therefore, if the sister group to the Eubrachyura lies somewhere within podotremes, the formation of the spermatheca, as a result of an invagination of two adjacent sternites, and the separate coxal position of the gonopores can be assumed to be the ancestral state of the brachyuran reproductive system (Hartnoll, 1969, 1979). However, as the coxal position of the gonopores in podotreme groups is a plesiomorphic character, it cannot be considered as an apomorphy (Scholtz & McLay, 2009).

Even though theories on the evolution of the female seminal receptacles in eubrachyurans have been a side-product in some studies, only two studies have thoroughly addressed this issue (Hartnoll, 1968; McLay & López-Greco, 2011). While Hartnoll (1968) focused on the morphological investigation of the female genital ducts, McLay & López-Greco (2011) took mating and growth characters as well as reproductive strategies into account. Authors of earlier studies used the term spermatheca instead of seminal receptacle (Jensen et al., 1996; López-Greco et al., 1999; Jennings et al., 2000; Lautenschlager et al., 2010; Becker et al., 2011). However, the spermatheca is made of two adjacent endosternites (7/8) that invaginate to different degrees. By contrast, the seminal receptacle lies in sternite 6. Therefore, a transformation from a spermatheca to a seminal receptacle would have involved the attachment of the laterally positioned oviduct to the medial side of the spermatheca as well as the segmental relocation of the spermatheca to the 6th sternite. On the other hand, the transition from the coxal gonopore to the sternal gonopore with the new formation of a seminal receptacle seems more likely because it "only" involved the transition of the oviduct from the coxa of the 6th thoracic appendage (= 3rd walking leg) to the sternite of the 6th thoracic segment and possibly an invagination of the cuticle around the gonopore opening. From a morphological point of view, a mixed use of the podotreme term "spermatheca" and the eubrachyuran term "seminal receptacle" would falsely imply

a common phylogenetic origin and should be avoided. The seminal receptacle is an apomorphy of the Eubrachyura and cannot be homologised with the spermatheca (Tavares & Secretan, 1993; Guinot & Quenette, 2005; McLay & López-Greco, 2011).

Since the oviduct might have connected to the cuticular gonopore on the 6th sternite, it can be assumed that the cuticle-adjoining oviduct orifice (character 1.1) is the ancestral state. In this case, the cuticle-distant oviduct orifice (character 1.2) has evolved multiple times, at least once in Portunoidea and once in Xanthidae.

The secretory tissue of the seminal receptacle probably originated from the oviduct. Different degrees of the extension of the tissue resulted in the secretory area of the seminal receptacle (characters 2.1-2.2). This would explain the diversity of tissue distributions (secretory vs. cuticle) within the heterotreme groups (Figs. 5.4,5.5). Interestingly, the formation of an additional mono-layered glandular epithelium (character 3.2) in some heterotremes such as portunoid taxa (Zara et al., 2014; Pardo et al., 2017) and Eriphiidae (George, 2004; de Souza et al., 2016; Farias et al., 2017) might indicate a closer affiliation of these groups. The mono-layered glandular epithelium in Platyxanthidae has a different position within the seminal receptacle and depends on the reproductive cycle of the female (Farias et al., 2017). This indicates that its formation happened at least two times in eubrachyurans.

The bursa (character 4.1) of some representatives of Dorippidae (Vehof et al., 2017, 2018) and Cancridae (Jensen et al., 1996) seems to be an apomorphy of each of these groups.

Hartnoll (1968) concluded that the "simple" type vagina (character 5.1) represents the plesiomorphic character state and accordingly interpreting the "concave" type (character 5.2) as derived. The female gonopore is crescent shaped in the Diogenidae (Hess & Bauer, 2002) and the Galatheidae (Kronenberger et al., 2004). Both taxa are representatives of the Anomala, the well-established sister group to the Brachyura (Scholtz & Richter, 1995; Shen et al., 2013). Additionally, Becker & Scholtz (2017) investigated the reproductive system of the podotreme Homoloidea. Interestingly, they found a muscular operculum at the (crescent shaped) gonopore.

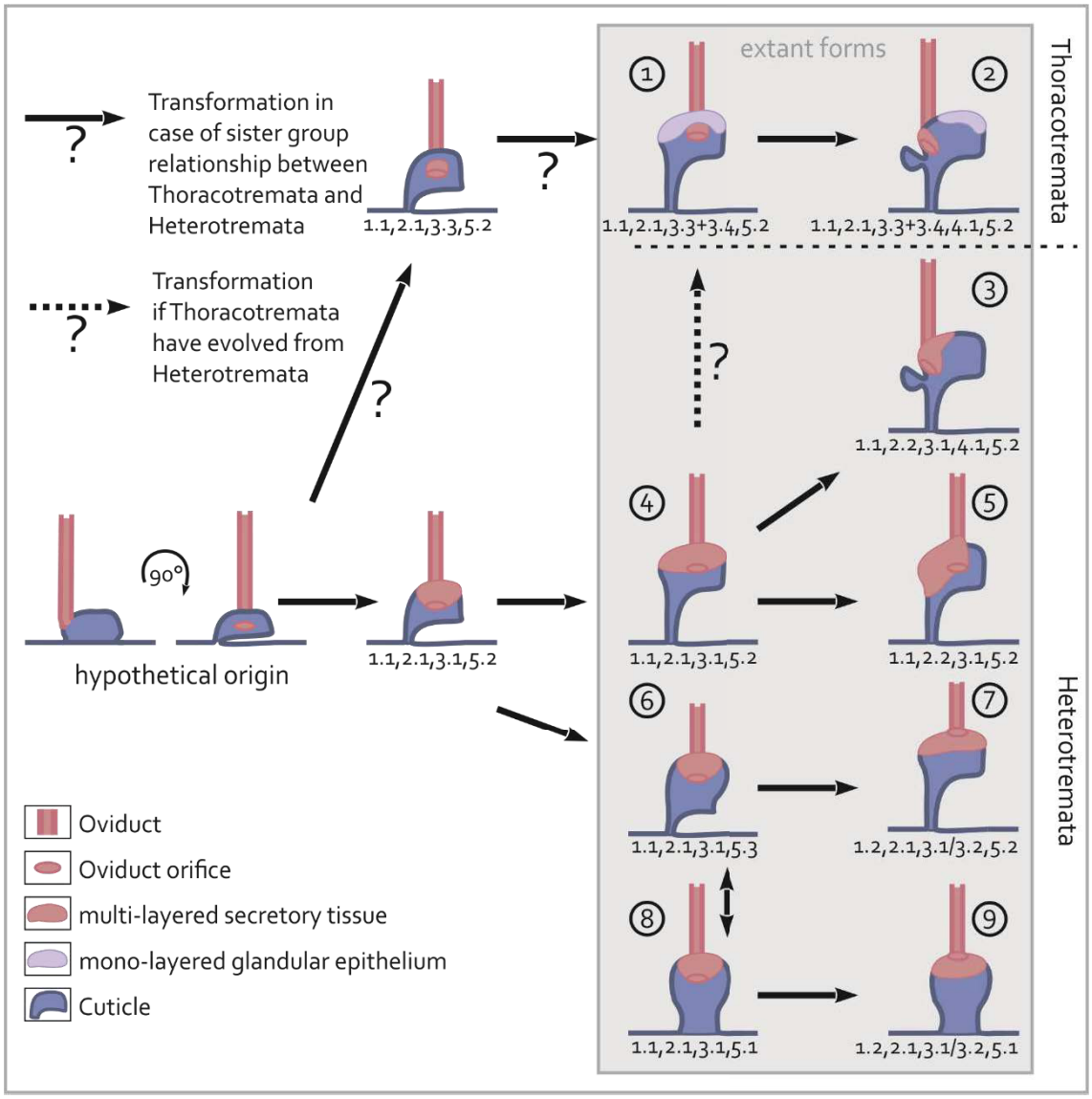


Fig. 5.5 Hypothetical evolutionary transformations of the eubrachyuran female reproductive system. The arrows with a continuous line indicate a transformation of character states in the case of a sister group relationship between monophyletic Heterotremata and Thoracotremata. The arrow with a dashed line indicates a transformation of the reproductive system if the Thoracotremata have evolved from paraphyletic Heterotremata. View from lateral. The external course of the oviduct along the medial side of the seminal receptacle is not shown. Ovaries not shown. If the hypothetical origin was an invagination of cuticle at the 6th sternite and the oviduct attached to this invagination, then different transformational pathways are possible. The extant forms underlain with grey are: **thoracotreme** - (**1**) and (**2**) with cuticle-adjoining oviduct orifice, crescent-shaped vagina, e.g. Cryptochiridae (Vehof et al., 2017), some with bursa, e.g. Percnidae and Hymenosomatidae (Kienbaum et al., 2018a,b: chapter 3, 4); **heterotreme** - (**3**) cuticle-adjoining oviduct orifice, crescent-shaped vagina, bursa, e.g. Dorippidae (Vehof et al., 2018); (**4**) and (**5**) cuticle-adjoining oviduct orifice, crescent-shaped vagina, e.g. Majoidea (Kienbaum et al., 2017: chapter 2); (**6**) cuticle-adjoining oviduct orifice, round and crescent-shaped vagina, e.g. Calappidae (Ewers-Saucedo et al., 2015); (**7**) cuticle-distant oviduct orifice, crescent-shaped vagina, e.g. Platyxanthidae (Farias et al., 2017); (**8**) cuticle adjoining oviduct orifice, round-vagina, e.g. Cancridae (Jensen et al., 1996) or (**9**) cuticle-distant oviduct orifice, round-vagina, e.g. Portunidae (Zara et al., 2014). 1.1 – 5.3 refer to the female character states.

Therefore, the crescent shape of the gonopore possibly reflects the ancestral state of eubrachyurans and the assumption of a plesiomorphic round vagina (without an operculum) seems unlikely. Moreover, it does not take into account the distribution of the remaining and enormously diverse female reproductive characters within a eubrachyuran tree (Figs. 5.4 – 5.7). It seems possible that early diverging eubrachyurans had a short crescent-shaped vagina due to an invagination of the cuticle that was closed by an operculum (Fig. 5.5). This type of vagina could have undergone a transformation into a round vagina and the loss of the operculum as found in Cancridae (Orensanz et al., 1995; Jensen et al., 1996; Pardo et al., 2013), Menippidae (de Souza et al., 2017) and Portunoidea (Hartnoll, 1968; Zara et al., 2014; de Souza et al., 2017; Pardo et al., 2017). A close affiliation of Portunoidea and Cancroidea (Schubart & Reuschel, 2009) but not necessarily Menippidae (as part of the Eriphioidea), indicates the convergent evolution of this character within eubrachyurans. The vagina consisting of both a crescent-shaped area and a round area (character 5.3) as found in Calappidae (Ewers-Saucedo et al., 2015) and Eriphiidae (George, 2004) also evolved independently in these groups. The existence of both, the round vagina (Menippidae) and crescent/round vagina (Eriphiidae) in the Eriphioidea emphasises the possibility of a convergent evolution of the vagina types.

In contrast to the heterotreme female reproductive system, the one of thoracotremes is more uniform. The mono-layered glandular epithelium (character 3.4) that lines the seminal receptacle has probably evolved once within thoracotremes and represents an apomorphy. Even though it might be the same type of epithelium as is present in some heterotreme representatives, it should not be homologised because the position and extension of these epithelia is different to the thoracotreme. Contrarily, the thoracotreme holocrine transfer tissue (sensu Becker et al., 2011) (character 3.3) can be homologised with the heterotreme multi-layered secretory tissue due to its structural properties and position at the oviduct orifice (McLay & Becker, 2015; Kienbaum et al., 2018a: chapter 3). However, it remains unclear whether the varying extent of secretory tissue in the seminal receptacle of heterotreme species has been reduced to the oviduct orifice in thoracotremes or if it did not extend in the ancestral lineage in the first place. Within the monophyletic Thoracotremata, the Ocypodoidea have a longitudinal division of the cuticle and the tissue within the seminal receptacle (character 2.2). Additionally, another type of secretory tissue lines the seminal receptacle (character 3.5) (Sant'Anna et al., 2007; López-Greco et al., 2009; Lautenschlager et al., 2010). Both character states set them apart from the remaining thoracotremes and could be interpreted as apomorphies. Unfortunately, the polyphyletic status of Ocypodoidea challenges this assumption (Tsang et al., 2014; Basso et al., 2017). The structurally very similar reproductive systems of the herein investigated *P. gibbesi* (Kienbaum et al., 2018a: chapter 3) and *L. naiyanetri* (Klaus et al., 2014; Kienbaum et al., 2018b:

chapter 4) in combination with the existence of a bursa (character 4.1) might have evolved once in a common ancestor of Percnidae and Hymenosomatidae.

The following scenarios of the evolution of the reproductive systems in Eubrachyura describe only a section of possible variants.

In *scenario 1*, the character states (1.1 – 5.3) of the female reproductive system are mapped on the phylogenetic tree of Basso et al. (2017). Basso et al. (2017) is used because it is exemplary for molecular studies of brachyuran phylogeny such as Tang et al. (2017a, b) and Yuhui et al. (2017) with very similar results. Some of the eubrachyuran species in which the reproductive system has been studied are not part of this phylogenetic tree. On the other hand, many groups that lack data on reproductive systems are included. This challenges the interpretation of a possible evolutionary scenario.

Scenario 2 is based on current phylogenetic hypotheses and on parsimonious principles. It describes one possibility of the evolutionary transformations of the reproductive systems of the investigated eubrachyurans.

(See table 5.1. for references.)

SCENARIO 1 (FIG. 5.6)

The ancestral state of the female reproductive system is assumed to present a cuticle-adjoining oviduct orifice (character 1.1). The dorsal area of the seminal receptacle (character 2.1) is lined by the multi-layered secretory tissue (character 3.1) and the vagina is crescent-shaped (character 5.2).

These characters are at least present in the majoid taxa that resolved as a monophyletic group. Since they represent plesiomorph characters they cannot be used to support this result. Two character states, the additional formation of a mono-layered glandular epithelium as "atypical columnar cells" or "modified dorsal epithelium" (character 3.2) and the round vagina (character 5.1), support the formation of a monophyletic group of Portunidae, Geryonidae, Matutidae, Leucosiidae and Menippidae. The monophyletic group of Portunidae and Geryonidae are supported by the cuticle-distant oviduct orifice (character 1.2) in both groups. This character state has evolved convergently in Xanthidae as well. The multi-layered secretory tissue is reduced in Menippidae. The Leucosiidae and Matutidae resolved as a well-supported monophyletic group. The mono-layered glandular epithelium must have been reduced in the seminal receptacle of Leucosiidae, that is only lined by the multi-layered secretory tissue (character 3.1), while the vagina is crescent shaped (character 5.2). These character states, if present in Matutidae as well, could support the monophyly of Leucosiidae and Matutidae. On the other hand, the leucosiid character states could also present apomorphies of this group alone. Unfortunately, there is no data on the reproductive systems of Matutidae that could

provide additional information. The heterotreme Potamidae are resolved as the sister group of the thoracotremes. This implies paraphyletic Heterotremata. Unfortunately, data on the female reproductive systems of Potamidae is missing and shared character states of Potamidae and thoracotreme taxa that would support such a relationship have yet to be found. The mono-layered glandular epithelium that lines the seminal receptacle as well as the holocrine transfer tissue (character 3.3+3.4) are character states that support monophyletic Thoracotremata. The conformity of the character states in thoracotremes provide only limited information about evolutionary transformations. Some Ocypodidae possess a different type of secretory tissue (character 3.5). This character state might be adequate to be compared between the polyphyletic ocypodoid taxa in order to resolve their phylogenetic position.

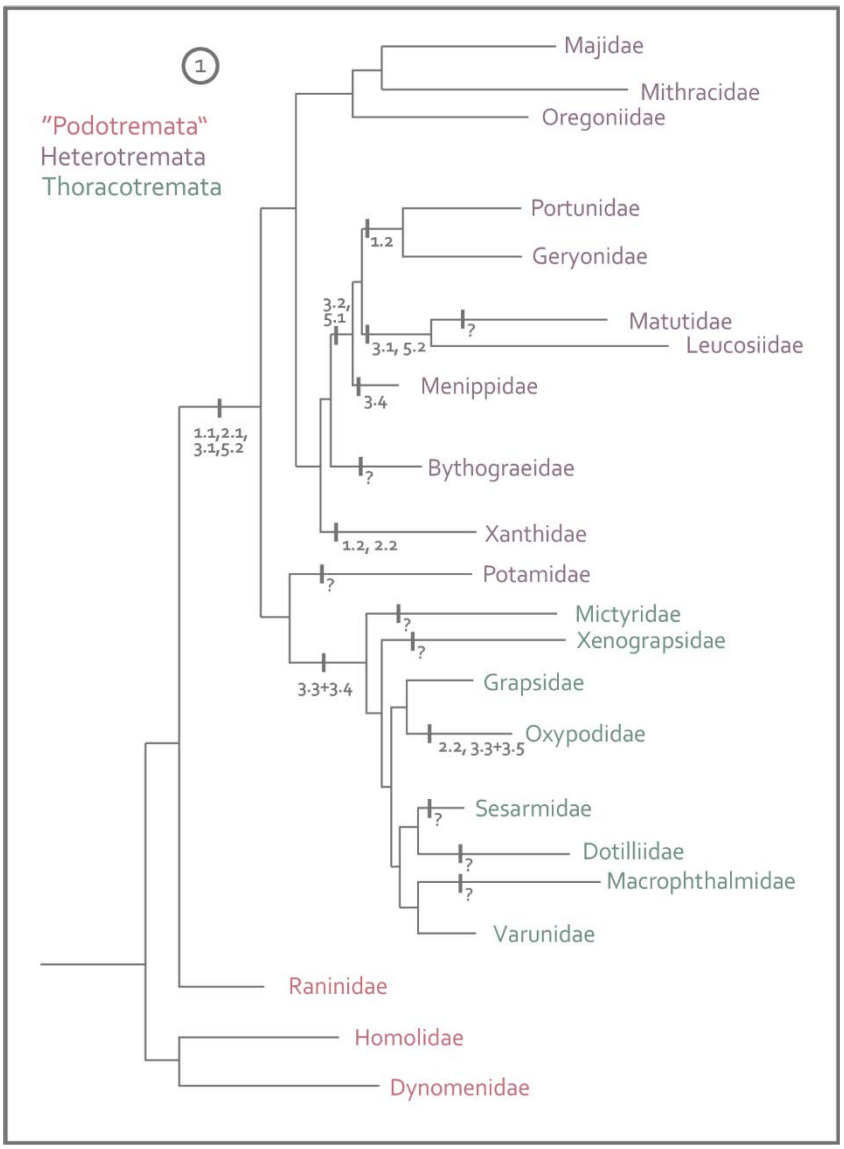


Fig. 5.6 Scenarios of the evolution of the reproductive systems in Eubrachyura. (1) The described character states (1.1 – 5.3) of the female reproductive system (Fig. 5.4) are mapped on a phylogenetic tree modified from Basso et al. (2017). Please notice, that of the described character complexes, only one character state can occur within a species at a time (e.g. 5.1 *or* 5.2 *or* 5.3). Reductions are not mentioned separately.

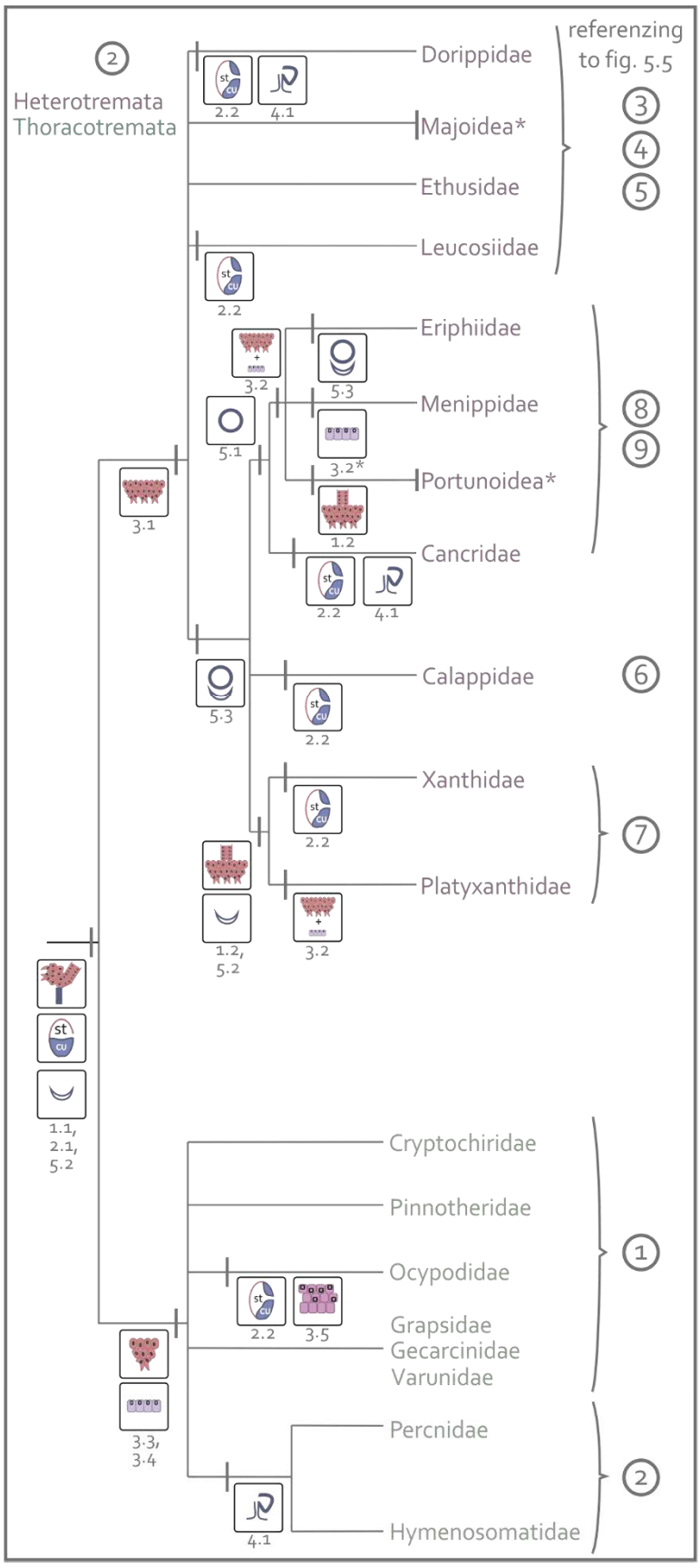


Fig. 5.7 Scenario 2 of the evolution of the reproductive systems in Eubrachyura based on current phylogenetic hypotheses in comparison with parsimonious considerations. Please notice, that of the described character complexes, only one character state can occur within a species at a time (e.g. 5.1 *or* 5.2 *or* 5.3). Reductions are not mentioned separately.

The encircled numbers 1 - 9 correspond to the extant forms that are shown in fig. 5.5.

SCENARIO 2 (Fig. 5.7):

The ancestral state of the female reproductive system presents equally to scenario 1 the cuticle-adjoining oviduct orifice (character 1.1) and the crescent shaped vagina (character 5.2) (Fig. 5.5).

The dorsal area of the seminal receptacle is lined by the multi-layered secretory tissue (character 2.1, 3.1) in all heterotreme taxa.

The Majoidea are widely accepted as monophyletic (Hultgreen & Stachowicz, 2008; Mahon & Neigel, 2008) and their position as an early diverging group within the heterotreme tree (Spears et al., 1992; Jamieson et al., 1995; Porter et al., 2005; Basso et al., 2017 - scenario 1) is here supported by the similarities of the reproductive system with the ancestral state. A close affiliation of Majoidea and Ethusidae has been suggested by Bracken et al. (2009). The similar character states of the reproductive system would support such a relationship. Unfortunately, the absence of apomorphic characters in the reproductive system of Majoidea and Ethusidae leads to unresolved relationships between these groups. Sin et al. (2009) supports the sister group relationship of Ethusidae and Dorippidae, that constitute the Dorippoidea. Dorippid taxa have a bursa (character 4.1) and in some species a very intriguing female reproductive system that differs from the Ethusidae. This could be interpreted as an apomorphy of the Dorippidae but is unfortunately not informative concerning possible sister group relationships.

The position of Leucosiidae within the heterotreme tree is still debated (Chu et al., 2009; Tsang et al., 2014; Basso et al., 2017 - scenario 1). The similarities of the female reproductive system with the ancestral state argue in favour of an early diverging lineage. However, a probable sister group relationship remains unresolved too.

Based on the formation of a vagina with an inwards round shape associated with the shortening of the outwards crescent shaped part and the resulting formation of an operculum (character 5.3), all remaining investigated heterotremes could form a monophyletic group.

Even though its internal relationships are unsettled (Martin & Davis, 2001; Karasawa et al., 2008; Ng et al., 2008) the Portunoidea are probably monophyletic (Bracken et al., 2009; Tsang et al., 2014; Basso et al., 2017). A close relationship of Portunoidea and Cancroidea has been discussed by Schubart & Reuschel (2009). By contrast, the Eriphioidea and its constituting groups, the Menippidae, the Eriphiidea and the Platyxanthidae (Ng., et al. 2008), have been resolved as polyphyletic (Tsang et al. 2014; Lay et al. 2014).

Based on these phylogenetic hypotheses, the reproductive characters are interpreted as follows. All investigated Portunoidea, the Cancridae and the Menippidae represent heterotreme taxa with a round vagina and the associated loss of an operculum (character 5.1). This could represent an apomorphy.

The presence of the mono-layered glandular epithelium (character 3.2) supports a group of the Portunoidea, the Eriphiidae and the Menippidae (the latter two belonging to the Eriphioidea). The Portunoidea have a cuticle-distant oviduct orifice (character 1.2). The Eriphiidae have a vagina that is inwards round shaped and outwards crescent shaped (character 5.3) and in the Menippidae, the multi-layered secretory tissue is reduced and the mono-layered glandular epithelium lines the seminal receptacle (character 3.2*). Unfortunately, the absence of apomorphic characters in the reproductive system of Eriphiidae and Menippidae that would support their affiliation to Eriphioidea, lead to unresolved relationships between these groups.

Taxa of the Calappoidea resolved as paraphyletic in Bracken et al. (2009) and as polyphyletic in Tsang et al. (2014), with varying sister group relationships. The character distribution in the investigated species provide no useful information to resolve this problem.

The Xanthoidea are not monophyletic (Lay et al., 2011) and the Platyxanthidae are part of the polyphyletic Eriphioidea (Ng et al., 2008). The difference of character states in Eriphiidae, Menippidae and Platyxanthidae support a polyphyletic status of Eriphioidea. On the other hand, based on the formation of the cuticle distant oviduct orifice (character 1.2) and the crescent shaped vagina (character 5.2) a sister group relationship of the Xanthidae and Platyxanthidae could be supported. The mono-layered glandular epithelium in the seminal receptacle of Platyxanthidae can be interpreted as an apomorphy of this group (character 3.2).

The monophyly of all Thoracotremata (Jamieson et al., 1995; von Sternberg & Cumberlidge, 2001; Tsang et al., 2014) is supported by the character states of their reproductive system. The multi-layered secretory tissue is restricted to the oviduct orifice (holocrine transfer tissue) (character 3.3) and the mono-layered glandular epithelium (character 3.4) lines the dorsal area of the seminal receptacle (character 2.1). To date it is not possible to ascertain whether the multi-layered tissue of the heterotreme seminal receptacle has been reduced or whether it didn't expand in the thoracotreme lineage. Due to the homogeneity of the female reproductive system, the character states provide only limited information about evolutionary transformations. Interestingly, the Ocypodidae might be separated from the remaining thoracotreme species based on the existence of a different kind of multi-layered tissue (character 3.5) that is present in some investigated species. On the other hand, the Ocypodoidea are probably polyphyletic and intermingle with grapsoid taxa (Chu et al., 2009; Tsang et al., 2014; Basso et al., 2017). Therefore, the character state distribution in the Ocypodidae is not sufficient to provide any more details.

At the moment, the Percnidae are assigned to the Grapsoidea, a group of uncertain phylogenetic status (Schubart et al., 2006; Schubart & Cuesta, 2010). The here investigated Percnidae (*P. gibbesi*. Kienbaum et al., 2018a: chapter 3) and Hymenosomatidae (*L. naiyanetri*, Kienbaum et al., 2018b: chapter 4) share very similar reproductive systems. This includes the presence of a bursa and an

oviduct that does not transit into the seminal receptacle directly but into a cuticular duct. This might be interpreted in favour of a sister group relationship.

At this point, the characters of the reproductive system of eubrachyuran females can be used to test the affiliation of eubrachyuran taxa to either Heterotremata or Thoracotremata with some certainty. This has been exemplified for *Limnopilos naiyanetri* (Kienbaum et al., 2018b: chapter 4). Especially the secretory tissues that line the seminal receptacle contain a useful phylogenetic signal. Additionally, some characters, as for example, the cuticle-distant oviduct orifice and the round vagina provide important information to confirm the affiliation of species to a certain group. However, without a reliable brachyuran phylogeny the evolutionary transformation of the female reproductive system is a challenging matter. The probability of multiple convergent transformations limits the applicability of these characters in the context of small-scale brachyuran phylogeny.

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6 | CONCLUSIONS

This work provides an investigation of the existing data on the morphology of eubrachyuran male copulatory and female reproductive systems. It challenges some previous hypotheses and argues about the usefulness of characters of the male copulatory and female reproductive system for the understanding of their evolutionary transformation. Additionally, it provides possible scenarios for the evolution of the reproductive system in Eubrachyura by the definition of characters that could also be used in future studies.

The investigation of the majoid species *Mithraculus sculptus* and *Stenorhynchus seticornis* challenges the hypothesis of the velum and its involvement in the fertilisation process. Additionally, it made clear that a sole division of the seminal receptacle into a dorsal and ventral chamber is a simplified characterisation. Both hypotheses might have obstructed the view on potential important characters by producing biased premises.

Percnon gibbesi as a representative of the thoracotreme Percnidae showed a new combination of characters in the female reproductive systems.

The female reproductive system of the hymenosomatid *Limnopilos naiyanetri* exemplifies, that these characters can contribute to phylogenetic investigations. Here, the combination of character states in males (for example: the sternal gonopore) and females (for example: the mono-layered epithelium in the seminal receptacle) led to the most probable answer of thoracotreme Hymenosomatidae.

Admittedly, none of the attempts to bring a system into the chaotic phylogeny of the Brachyura was successful, *yet* and the basis of this work remains a rag rug of data presented from different perspectives and different approaches. To date, it is not possible to find "the evolutionary scenario", because too many inconsistencies remain and too few species have been investigated.

With the data at hand, it cannot with certainty be decided whether the Heterotremata are monophyletic. The male gonopods and the female reproductive system are immensely diverse and might have evolved through multiple pathways. Unfortunately, for now it remains unsolved at what point these pathways diverged. At least, the defined character states provide a rough direction of how they might have evolved. By contrast, the thoracotreme gonopods and the sternal position of the gonopore as well as the consistent characters in the female system would argue in favour of their monophyletic status, but this too remains an assumption based on the existing data.

It needs additional efforts to compile the existing data as well as to create more data in great detail and with modern technology that might lead to the definition of additional characters. Today, the ongoing technological progress facilitates the use of high resolution microscopy or μCT-scans for 3-dimensional imaging within the intact body of the animal. Additionally, a combination of morphological data with a thorough molecular analysis might be of use. Even though, it is important

to find consistencies within the system, the impulse to simplify and unify data in order to customise them into given hypotheses might be misleading. Therefore, a careful evaluation and consideration of the data without any rash conclusions in future studies is indispensable.

7 | REFERENCES

Ahyong ST, Lai JCY, Sharkey D, Colgan DJ, Ng PKL. (2007). Phylogenetics of the brachyuran crabs (Crustacea: Decapoda): The status of Podotremata based on small subunit nuclear ribosomal RNA. *Molecular Phylogenetics and Evolution*, 45, 576-586.

Alcock A. (1900). Brachyura Catometopa or Grapsoidea: Materials for a carcinological fauna of India, No. 6. *Journal of the Asiatic Society of Bengal*, 69, 279-456.

Andrews EA. (1911). Male organs for sperm-transfer in the cray-fish, *Cambarus affinis*: their structure and use. *Journal of Morphology*, 22, 239-297.

Anilkumar G, Sudha K, Anitha E, Subramoniam T. (1996). Aspects of sperm metabolism in the spermatheca of the brachyuran crab *Metopograpsus messor* (Forskal). *Journal of Crustacean Biology*, 16, 310-314.

Antunes M, Zara FJ, López-Greco LS, Negreiros-Fransozo ML. (2016). Morphological analysis of the female reproductive system of *Stenorhynchus seticornis* (Brachyura: Inachoididae) and comparisons with other Majoidea. *Invertebrate Biology*, 135, 75-86.

Antunes M, Zara FJ, López-Greco LS, Negreiros-Fransozo ML. (2018). Male reproductive system of the arrow crab *Stenorhynchus seticornis* (Inachoididae). *Invertebrate Biology*, 137, 171-184.

Armstrong JH. (1988). Reproduction in the paddle crab *Ovalipes catharus* (Decapoda: Portunidae) from Blueskin Bay, Otago, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, 22, 529-536.

Baeza JA, Fernández M. (2002). Active brood care in *Cancer setosus* (Crustacea: Decapoda): the relationship between female behaviour, embryo oxygen consumption and the cost of brooding. *Functional Ecology*, 16, 241-251.

Basso A, Babbucci M, Pauletto M, Riginella E, Patarnello T, Negrisolo E. (2017). The highly rearranged mitochondrial genomes of the crabs *Maja crispata* and *Maja squinado* (Majidae) and gene order evolution in Brachyura. *Scientific Reports*, 7, 4096.

Bauer RT. (1976). Mating behaviour and spermatophore transfer in the shrimp *Heptacarpus pictus* (Stimpson) (Decapoda: Caridea: Hippolytidae). *Journal of Natural History*, 10, 415-440.

Bauer RT. (1986). Phylogenetic trends in sperm transfer and storage complexity in decapod crustaceans. *Journal of Crustacean Biology*, 6, 313-325.

Bauer RT. (1991). Sperm transfer and storage structures in penaeoid shrimps: a functional and phylogentic perspective. In: Bauer RT, Martin JW, editors. Crustacean sexual biology. New York: Columbia University Press. p 183-207.

Bawab FM, El-Sherief SS. (1988). Stages of the reproductive cycle of the female crab *Portunus pelagicus* (L., 1758) based on the anatomical changes of the spermatheca (Decapoda Brachyura, Portunidae). *Crustaceana*, 54, 139-148.

Becker C, Scholtz G. (2017). Phylogenetic implications of sperm storage in Podotremata: Histology and 3D-reconstructions of spermathecae and gonopores in female carrier crabs (Decapoda: Brachyura: Homoloidea). *Journal of Morphology*, 278, 89-105.

Becker C, Brandis D, Storch V. (2011). Morphology of the female reproductive system of European pea crabs (Crustacea, Decapoda, Brachyura, Pinnotheridae). *Journal of Morphology*, 272, 12-26.

Becker C, Türkay M, Brandis D. (2012). The male copulatory system of European pea crabs (Crustacea, Brachyura, Pinnotheridae). *Journal of Morphology*, 273, 1306-1318.

Benhalima K, Moriyasu M. (2001). Prevalence of bacteria in the spermathecae of female snow crab, *Chionoecetes opilio* (Brachyura: Majidae). *Hydrobiologia*, 449, 261-266.

Beninger PG, Larocque R. (1998). Gonopod tegumental glands: a new accessory sex gland in the Brachyura. *Marine Biology*, 132, 435-444.

Beninger PG, Elner RW, Foyle TP, Odense PH. (1988). Functional anatomy of the male reproductive system and the female spermatheca in the snow crab *Chionoecetes opilio* (O. Fabricius) (Decapoda: Majidae) and a hypothesis for fertilization. *Journal of Crustacean Biology*, 8, 322-332.

Beninger PG, Elner RW, Poussart Y. (1991). Gonopods of the majid crab *Chionoecetes opilio* (O. Fabricius). *Journal of Crustacean Biology*, 11, 217-228.

Beninger PG, Lanteigne C, Elner RW. (1993). Reproductive processes revealed by spermatophore dehiscence experiments and by histology, ultrastructure, and histochemistry of the female reproductive system in the snow crab *Chionoecetes opilio* (O. Fabricius). *Journal of Crustacean Biology*, 13, 1-16.

Beninger PG, Ferguson A, Lanteigne C. (1995). The gonopod tegumental glands of snow crab *Chionoectes opilio* (Fabricius, 1788) are accessory reproductive glands. *Journal of Shellfish Research*, 14, 365-370.

Bracken HD, Toon A, Felder DL, Martin JW, Finley M, Rasmussen J, Palero F, Crandall KA. (2009). The decapod tree of life: compiling the data and moving toward a consensus of decapod evolution. *Arthropod Systematics & Phylogeny*, 67, 99-116.

Brandis D, Storch V, Türkay M. (1999). Morphology and function of the copulatory system in freshwater crabs of the genus *Potamon*. *Journal of Morphology*, 239, 157-166.

Brösing A, Richter S, Scholtz G. (2006). Phylogenetic analysis of the Brachyura (Crustacea, Decapoda) based on characters of the foregut with establishment of a new taxon. *Journal of Zoological Systematics and Evolutionary Research*, 45, 20-32.

Castilho GG, Ostrensky A, Pie MR, Boeger WA. (2008). Morphology and histology of the male reproductive system of the mangrove land crab *Ucides cordatus* (L.)(Crustacea, Brachyura, Ocypodidae). *Acta Zoologica (Stockholm)*, 89, 157-161.

Castilho-Westphal GG, Ostrensky A, Pie MR, Boeger WA. (2013). Morphology of the female reproductive system and reproductive cycle of the mangrove land crab *Ucides cordatus* (L.) in the Baı´a de Antonina, Parana´, Brazil. *Acta Zoologica (Stockholm)*, 94, 86-93.

Castro P. (2000). Crustacea Decapoda: A revision of the Indo-west Pacific species of palicid crabs (Brachyura Palicidae). In: Crosnier A, editor. Résultats des campagnes MUSORSTOM, 21. *Mémoires du Muséum national d'Histoire naturelle*, *Paris*, 184. 437-610.

Cheung TS. (1968). Trans-molt retention of sperm in the female stone crab, *Menippe Mercenaria* (Say). *Crustaceana*, 15, 117-120.

Chu KH, Tsang LM, Ma KY, Chan TY, Ng PKL. (2009). Decapod phylogeny: what can protein-coding genes tell us? In: Martin JW, Crandall KA, Felder DL, editors. Crustacean Issues 18: Decapod Crustacean Phylogenetics. Boca Raton, Florida: Taylor & Francis/CRC Press. p 101-112.

Chuang CTN, Ng PKL. (1994). The ecology and biology of Southeast Asian false spider crabs (Crustacea: Decapoda: Brachyura: Hymenosomatidae). *Hydrobiologia*, 285, 85-92.

Cobo VJ. (2002). Breeding period of the arrow crab *Stenorhynchus seticornis* from Couves Island, south-eastern Brazilian coast. *Journal of the Marine Biological Association of the United Kingdom*, 82, 1031-1032.

Cochran DM. (1935). The skeletal musculature of the blue crab, *Callinectes sapidus* Rathbun. *Smithonian miscellaneous collections*, 92, 1-76.

Cronin LE. (1947). Anatomy and histology of the male reproductive system of *Callinectes sapidus* Rathbun. *Journal of Morphology*, 81, 209-239.

Crouau Y. (1997). Comparison of crustacean and insect mechanoreceptive setae. *International Journal of Insect Morphology and Embryology*, 26, 181-190.

Davie PJF, Guinot D, Ng PKL. (2015a). Anatomy and functional morphology of Brachyura. In: Castro P, Davie PJF, Guinot D, Schram FR, Von Vaupel Klein JC, editors. Treatise on Zoology - Anatomy, Taxonomy, Biology - The Crustacea, complementary to the volumes translated from the French of the Traité de Zoologie, 9C-I, Decapoda: Brachyura (Part 2). Leiden: Koninklijke Brill NV. 11-163.

Davie PJF, Guinot D, Ng PKL. (2015b). Phylogeny of Brachyura. In: Castro P, Davie PJF, Guinot D, Schram FR, Von Vaupel Klein JC, editors. Treatise on Zoology - Anatomy, Taxonomy, Biology - The Crustacea, complementary to the volumes translated from the French of the Traité de Zoologie, 9C-I, Decapoda: Brachyura (Part 2). Leiden: Koninklijke Brill NV. 921-979.

De Grave S, Pentcheff D, Ahyong ST. (2009). A classification of living and fossil genera of decapod crustaceans. *Raffles Bulletin of Zoology*, supplement no 21, 1-109.

de Saint-Laurent M. (1980). Sur la classification et la phylogénie des Crustacés Décapodes Brachyoures. I. Podotremata Guinot, 1977, et Eubrachyura sect. nov. *Comptes rendus hebdomadaires des Seances de l'Academie des Sciences, Paris,(D)*, 290, 1265-1268.

de Souza LP, Silva JRF. (2009). Morphology of the female reproductive system of the red-clawed mangrove tree crab (*Goniopsis cruentata* Latreille, 1803). *Scientia Marina (Barcelona)*, 73, 527-539.

de Souza LP, Silva JRF, Araujo AM, Camargo-Mathias MI. (2013). Morphology of the female genital ducts of the blue land crab *Cardisoma guanhumi* (Crustacea: Brachyura: Gecarcinidae). *Acta Zoologica (Stockholm)*, 94, 300-307.

de Souza LP, Ogawa CY, Silva JRF, Camargo-Mathias MI. (2017). Comparative morphology of the female genital ducts of seven eubrachyuran crabs (Saint Laurent, 1980). *Acta Zoologica (Stockholm)*, 98, 125-135.

Derby CD. (1989). Physiology of sensory neurons in morphologically identified cuticular sensilla of crustaceans. In: Felgenhauer BE, Watling L, Thistle AB, editors. *Functional morphology of feeding and grooming in Crustacea*. Rotterdam: AA Balkema. p 27-47.

Deudero S, Frau A, Cerda M, Hampel H. (2005). Distribution and densities of the decapod crab *Percnon gibbesi*, an invasive Grapsidae, in western Mediterranean waters. *Marine Ecology Progress Series*, 285, 151-156.

Diesel R. (1986). Optimal mate searching strategy in the symbiotic spider crab *Inachus phalangium* (Decapoda). *Ethology*, 72, 311-328.

Diesel R. (1989). Structure and function of the reproductive system of the symbiotic spider crab *Inachus phalangium* (Decapoda: Majidae): observations on sperm transfer, sperm storage, and spawning. *Journal of Crustacean Biology*, 9, 266-277.

Diesel R. (1990). Sperm competition and reproductive success in the decapod *Inachus phalangium* (Majidae): a male ghost spider crab that seals off rivals' sperm. *Journal of Zoology*, 220, 213-223.

Diesel R. (1991). Sperm competition and the evolution of mating behavior in Brachyura, with special reference to spider crabs (Decapoda, Majidae). In: Bauer RT, Martin JW, editors. Crustacean sexual biology. New York: Columbia University Press. p 145-163.

Elner RW, Gass CA, Campbell A. (1985). Mating behavior of the jonah crab, *Cancer borealis* Stimpson (Decapoda, Brachyura). *Crustaceana*, 48, 34-39.

Ewers-Saucedo C, Hayer S, Brandis D. (2015). Functional morphology of the copulatory system of box crabs with long second gonopods (Calappidae, Eubrachyura, Decapoda, Crustacea). *Journal of Morphology*, 276, 77-89.

Ewers-Saucedo C, Wares JP, Hanel R, Brandis D. (2016). Evolution of male copulatory organs in box crabs (Decapoda: Eubrachyura: Calappidae De Haan, 1833). *Journal of Crustacean Biology*, 36, 804-814.

Farias NE, Spivak ED, Luppi TA. (2017). Functional morphology of the female reproductive system of a crab with highly extensible seminal receptacles and extreme sperm storage capacity. *Journal of Morphology*, 278, 919-935.

Feldmann RM, McLay CL. (1993). Geological history of brachyuran decapods from New Zealand. *Journal of Crustacean Biology*, 13, 443-455.

Garcia TM, Silva JRF. (2006). Testis and vas deferens morphology of the red-clawed mangrove tree crab (*Goniopsis cruentata*)(Latreille, 1803). *Brazilian Archives of Biology and Technology*, 49, 339-345.

Garm A, Høeg JT. (2006). Ultrastructure and functional organization of mouthpart sensory setae of the spiny lobster *Panulirus argus*: New features of putative mechanoreceptors. *Journal of Morphology*, 267, 464-476.

Garth JS. (1958). Brachyura of the pacific coast of America, Oxyrhyncha. *Allan Hancock Pacific Expeditions*, 21, 1-854.

George S. (2004). Untersuchungen zu Bau und Funktion der Fortpflanzungsorgane von Eriphia verrucosa (Forskål 1775) als Beitrag zum Verständnis von Taxonomie und Ökologie von xanthoiden Krabben. [Diploma Thesis] Frankfurt am Main: Johann Wolfgang Goethe Universität. 1-119.

González-Pisani X, Barón P, López-Greco LS. (2011). Functional anatomy of the female reproductive systems of two spider crabs (Decapoda, Majoidea). *Invertebrate Biology*, 131, 61-74.

Guinot D. (1977). Propositions pour une nouvelle classification des Crustacés Décapodes Brachyoures. *Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences Serie D*, 285, 1049-1052.

Guinot D. (2011). The position of the Hymenosomatidae MacLeay, 1838, within the Brachyura (Crustacea, Decapoda). *Zootaxa*, 2890, 40-52.

Guinot D, Richer de Forges B. (1997). Affinités entre les Hymenosomatidae MacLeay, 1838 et les Inachoididae Dana, 1851 (Crustacea, Decapoda, Brachyura). *Zoosystema,* 19, 453-502.

Guinot D, Bouchard J-M. (1998). Evolution of the abdominal holding systems of brachyuran crabs (Crustacea, Decapoda, Brachyura). *Zoosystema*, 20, 613-694.

Guinot D, Hurtado LA. (2003). Two new species of hydrothermal vent crabs of the genus *Bythograea* from the southern East Pacific Rise and from the Gelepagos Rift (Crustacea Decapoda Brachyura Bythograeidae). *Comptes Rendus Biologies*, 326, 423-439.

Guinot D, Quenette G. (2005). The spermatheca in podotreme crabs (Crustacea, Decapoda, Brachyura, Podotremata) and its phylogenetic implications. *Zoosystema*, 27, 267-342.

Guinot D, Tavares M, Castro P. (2013). Significance of the sexual openings and supplementary structures on the phylogeny of brachyuran crabs (Crustacea, Decapoda, Brachyura), with new nomina for higher-ranked podotreme taxa. *Zootaxa*, 3665, 1-414.

Gurney R. (1938). Notes on some Decapod Crustacea from the red Sea: VI.-VIII. *Proceedings of the Zoological Society of London*, 108, Series B – Systematic and Morphological, 73-84.

Hard WL. (1942). Ovarian growth and ovulation in the mature blue crab, *Callinectes sapidus* Rathbun. *Chesapeake Biological Laboratory*, 46, 1-17.

Hartnoll RG. (1968). Morphology of the genital ducts in female crabs. *Zoological Journal of the Linnean Society*, 47, 279-300.

Hartnoll RG. (1969). Mating in the Brachyura. *Crustaceana*, 16, 161-181.

Hartnoll RG. (1979). The phyletic implications of spermathecal structure in the Raninidae (Decapoda: Brachyura). *Journal of Zoology*, 187, 75-83.

Hayer S, Schubart CD, Brandis D. (2015). Morphology and function of the female reproductive system of *Ebalia tumefacta* (Decapoda, Brachyura, Leucosiidae). *Journal of Morphology*, 276, 517-525.

Hayer S, Köhnk S, Boretius S, Brandis D. (2016). Ever more complex: a new type of organization of reproductive organs in female *Dorippe sinica* Chen, 1980 (Decapoda: Brachyura: Dorippidae). *Zoology*, 119, 455-463.

Hayer S, Köhnk S, Schubart CD, Boretius S, Gorb SN, Brandis D. (2017). Comparative study of the morphology of the female seminal receptacles of *Ilia nucleus* and *Persephona mediterranea* (Decapoda, Brachyura, Leucosiidae). *Arthropod structure & development*, 46, 274-286.

Hess GS, Bauer RT. (2002). Spermatophore transfer in the hermit crab Clibanarius vittatus (Creustacea, Anomura, Diogenidae). *Journal of Morphology*, 253, 166-175.

Hines AH, Jivoff PR, Bushmann PJ, van Montfrans J, Reed SA, Wolcott DL, Wolcott TG. (2003). Evidence for sperm limitation in the blue crab, *Callinectes sapidus*. *Bulletin of Marine Science*, 72, 287-310.

Hinsch GW, Cone MV. (1969). Ultrastructural observations of vitellogenesis in the spider crab, *Libinia emarginata* L. *Journal of Cell Biology*, 40, 336-342.

Hultgren KM, Stachowicz JJ. (2008). Molecular phylogeny of the brachyuran crab superfamily Majoidea indicates close congruence with trees based on larval morphology. *Molecular Phylogenetics and Evolution*, 48, 986-996.

Hultgren KM, Guerao G, Marques FPL, Palero FP. (2009). Assessing the contribution of molecular and larval morphological characters in a combined phylogenetic analysis of the superfamily Majoidea. In: Martin JW, Crandall KA, Felder DL, editors. Crustacean Issues 18: Decapod Crustacean Phylogenetics. Boca Raton, Florida: Taylor & Francis / CRC Press. p 437-455.

Ilan M, Shlagman A, Goren L, Shema T, Galil BS. (2015). A population of *Percnon gibbesi* (H. Milne Edwards, 1853)(Crustacea: Decapoda: Plagusiidae) along the Israeli coastline, southeast Mediterranean. *BioInvasions Records*, 4, 289-291.

Jamieson BGM, Tudge CC. (2000). Crustacea - Decapoda. In: Jamieson BGM, Adiyodi KG, Adiyodi RG, editors. Reproductive biology of invertebrates, 9C, Progress in male gamete ultrastructure and phylogeny. Chichester: John Wiley and Sons. 1-95.

Jamieson BGM, Guinot D, Richer de Forges B. (1995). Phylogeny of the Brachyura (Crustacea, Decapoda): evidence from spermatozoal ultrastructure. *Memoires du Museum national d'Histoire naturelle (France)*, 166, 265-283.

Jennings AC, McLay CL, Brockerhoff AM. (2000). Mating behaviour of *Macrophthalmus hirtipes* (Brachyura: Ocypodidae). *Marine Biology*, 137, 267-278.

Jensen PC, Bentzen P. (2012). A molecular dissection of the mating system of the Dungeness crab, *Metacarcinus magister* (Brachyura: Cancridae). *Journal of Crustacean Biology*, 32, 443-456.

Jensen PC, Orensanz JM, Armstrong DA. (1996). Structure of the female reproductive tract in the Dungeness crab (*Cancer magister*) and implications for the mating system. *The Biological Bulletin*, 190, 336-349.

Jivoff P. (1997). Sexual competition among male blue crab, *Callinectes sapidus*. *The Biological Bulletin*, 193, 368-380.

Johnson PT. (1980). Histology of the blue crab *Callinectes sapidus* - A model for the Decapoda. New York: Praeger Publishers. 1-440.

Karasawa H, Schweitzer CE. (2006). A new classification of the Xanthoidea sensu lato (Crustacea Decapoda: Brachyura) based on phylogenetic analysis and traditional systematics and evaluation of all fossil Xanthoidea sensu lato. *Contributions to Zoology*, 75, 23-73.

Karasawa H, Schweitzer CE, Feldmann RM. (2008). Revision of Portunoidea (Decapoda: Brachyura) with emphasis on the fossil genera and families. *Journal of Crustacean Biology*, 28, 82-127.

Karasawa H, Schweitzer CE, Feldmann RM. (2011). Phylogenetic analysis and revised classification of podotrematous Brachyura (Decapoda) including extinct and extant families. *Journal of Crustacean Biology*, 31, 523-565.

Keiler J, Wirkner CS, Richter S. (2017). One hundred years of carcinization – the evolution of the crab-like habitus in Anomura (Arthropoda: Crustacea). *Biological Journal of the Linnean Society*, 121, 200-222.

Kienbaum K, Scholtz G, Becker C. (2017). The morphology of the male and female reproductive system in two species of spider crabs (Decapoda: Brachyura: Majoidea) and the issue of the velum in majoid reproduction. *Arthropod Systematics & Phylogeny*, 75, 245-260.

Kienbaum K, Scholtz G, Becker C. (2018a). The morphology of the reproductive system in the crab *Percnon gibbesi* (Decapoda: Brachyura: Grapsoidea) reveals a new combination of characters in Thoracotremata. *Journal of Morphology*, 279, 883-894.

Kienbaum K, Vehof J, Becker C, Scholtz G. (2018b). The reproductive system of *Limnopilos naiyanetri* indicates a thoracotreme affiliation of Hymenosomatidae (Decapoda, Eubrachyura). *Arthropod structure & development*, 47, 513-520

Klaus S, Schubart CD, Brandis D. (2006). Phylogeny, biogeography and a new taxonomy for the Gecarcinucoidea Rathbun, 1904 (Decapoda: Brachyura). *Organisms, Diversity & Evolution*, 6, 199-217.

Klaus S, Goh GH, Malkowsky Y, Becker C, Plath M. (2014). Seminal receptacle of the pill box crab *Limnopilos naiyanetri* Chuang and Ng, 1991 (Brachyura: Hymenosomatidae). *Journal of Crustacean Biology*, 34, 407-411.

Krol RM, Hawkins WE, Overstreet RM. (1992). Reproductive components. In: Harrison FW, Humes AG, editors. Microscopic Anatomy of Invertebrates Decapod Crustacea. New York: Wiley-Liss, Inc. p 295-343.

Kronenberger K, Brandis D, Türkay M, Storch V. (2004). Functional morphology of the reproductive system of *Galathea intermedia* (Decapoda: Anomura). *Journal of Morphology*, 262, 500-516

Lai JCY, Mendoza JCE, Guinot D, Clark PF, Ng PKL. (2011). Xanthidae MacLeay, 1838 (Decapoda: Brachyura: Xanthoidea) systematics: a multi-gene approach with support from adult and zoeal morphology. *Zoologischer Anzeiger - A Journal of Comparative Zoology*, 250, 407-448.

Lai JCY, Thoma BP, Clark PF, Felder DL, Ng PKL. (2014). Phylogeny of eriphioid crabs (Brachyura, Eriphioidea) inferred from molecular and morphological studies. *Zoologica Scripta*, 43, 52-64.

Lanteigne C, Beninger PG, Gionet C. (1996). Ontogeny of female primary sexual characters in the majid crabs *Chionoecetes opilio* and *Hyas coarctatus*. *Journal of Crustacean Biology*, 16, 501-514.

Latreille PA. (1802). Histoire Naturelle, Générale et Particulière des Crustacés et des Insectes. Ouvrage faisant suite à l'Histoire Naturelle Générale et Particulière, composée par Leclerc de Buffon, et Rédigée par C. S. Sonnini, Membre de Plusieurs Sociétés Savantes. Famille naturelles des genres. Paris: F. Dufart. Tome troisième. xii + 467 pp., Tome quatrième. 387 pp., pls. 416-437.

Lautenschlager AD, Brandis D, Storch V. (2010). Morphology and function of the reproductive system of representatives of the genus Uca. *Journal of Morphology*, 271, 1281-1299.

Lee T-H, Yamazaki F. (1990). Structure and function of a special tissue in the female genital ducts of the Chinese freshwater crab *Eriocheir sinensis*. *The Biological Bulletin*, 178, 94-100.

Locke M. (2001). The Wigglesworth Lecture: Insects for studying fundamental problems in biology. *Journal of Insect Physiology*, 47, 495-507.

López-Greco LS, López GC, Rodríguez EM. (1999). Morphology of spermathecae in the estuarine crab *Chasmagnathus granulata* Dana 1851 (Grapsidae, Sesarminae). *Journal of Zoology*, 249, 469-493.

López-Greco LS, Fransozo V, Negreiros-Fransozo ML, Dos Santos DC. (2009). Comparative morphology of the seminal receptacles of *Ocypode quadrata* (Fabricius, 1787)(Brachyura, Ocypodoidea). *Zootaxa*, 2106, 41-50.

Lucas JS. (1971). The larval stages of some australian species of *Halicarcinus* (Crustacea, Brachyura, Hymenosomatidae). I. Morphology. *Bulletin of Marine Science*, 21, 471-490.

Lucas JS. (1980). Spider crabs of the family Hymenosomatidae (Crustacea; Brachyura) with particular reference to Australian species: systematics and biology. *Records of the Australian Museum*, 33, 148-247.

Lucas JS, Davie PJF. (1982). Hymenosomatid crabs of Queensland estuaries and tidal mud flats, including descriptions of four new species of *Elamenopsis* A. Milne-Edwards and a new species of *Amarinus* Lucas. *Memoirs of the Queensland Museum*, 20, 401-419.

Luque J. (2015). The oldest higher true crabs (Crustacea: Decapoda: Brachyura): insights from the early cretaceous of the Americas. *Palaeontology*, 58, 251-263.

Luque J, Schweitzer CE, Santana W, Portell RW, Vega FJ, Klompmaker AA. (2017). Checklist of fossil decapod crustaceans from tropical America. Part I: Anomura and Brachyura. *Nauplius*, 25, e2017025, 1-85.

Mahon BC, Neigel JE. (2008). Utility of arginine kinase for resolution of phylogenetic relationships among brachyuran genera and families. *Molecular Phylogenetics and Evolution*, 48, 718-727.

Martin JW, Abele LG. (1986). Notes on male pleopod morphology in the brachyuran crab family Panopeidae Ortmann, 1893, sensu Guinot (1978) (Decapoda). *Crustaceana*, 50, 182-198.

Martin JW, Davis GE. (2001). An updated classification of the recent Crustacea. *Science series*, *Natural History Museum of Los Angeles County*, 39, 1-124.

Masly JP. (2011). 170 years of "lock-and-key": genital morphology and reproductive isolation. *International Journal of Evolutionary Biology*, 2012, 1-10.

Mateos M, Hurtado LA, Santamaria CA, Leignel V, Guinot D. (2012). Molecular systematics of the deep-sea hydrothermal vent endemic brachyuran family Bythograeidae: a comparison of three Bayesian species tree methods. *PloS one*, 7, e32066.

McLay CL. (1988). Brachyura and crab-like Anomura of New Zealand. University of Auckland Marine Laboratory. 1-463.

McLay CL, López-Greco LS. (2011). A hypothesis about the origin of sperm storage in the Eubrachyura, the effects of seminal receptacle structure on mating strategies and the

evolution of crab diversity: How did a race to be first become a race to be last? *Zoologischer Anzeiger - A Journal of Comparative Zoology*, 250, 378-406.

McLay CL, Becker C. (2015). Reproduction in Brachyura. In: Castro P, Davie PJF, Guinot D, Schram FR, von Vaupel Klein JC, editors. Treatise on Zoology - Anatomy, Taxonomy, Biology - The Crustacea, complementary to the volumes translated from the French of the Traité de Zoologie, 9C-I, Decapoda: Brachyura (Part 1). Leiden: Koninklijke Brill NV. 185-243.

McLay CL, Sal Moyano MP. (2016). Calcium levels in the vulvar opercula of grapsoid and ocypodoid crabs (Decapoda: Brachyura). *Journal of Crustacean Biology*, 36, 220-228.

Minagawa M. (1993). Gonopods of the red frog crab *Ranina ranina* Linnaeus (Decapoda: Raninidae). *Crustacean research*, 22, 45-54.

Minagawa M, Chiu J-R, Kudo M, Takashima F. (1994). Male reproductive biology of the red frog crab, *Ranina ranina*, off Hachijojima, Izu Islands, Japan. *Marine Biology*, 118, 393-401.

Moriyasu M, Benhalima K, Duggan D, Lawton P, Robichaud D. (2002). Reproductive biology of male Jonah crab, *Cancer borealis* Stimpson, 1859 (Decapoda, Cancridae) on the Scotian shelf, northwestern Atlantic. *Crustaceana*, 75, 891-913.

Mueller C. (2001). First record of *Percnon gibbesi* (Crustacea: Brachyura: Grapsidae) for the Balearic Islands. *Senckenbergiana maritima*, 31, 83-89.

Naderloo R. (2011). Grapsoid crabs (Decapoda: Brachyura: Thoracotremata) of the Persian Gulf and the Gulf of Oman. *Zootaxa*, 3048, 1-43.

Naderloo R, Schubart CD. (2010). Description of a new species of Parasesarma (Crustacea; Decapoda; Brachyura; Sesarmidae) from the Persian Gulf, based on morphological and genetic characteristics. *Zoologischer Anzeiger - A Journal of Comparative Zoology*, 249, 33-43.

Neumann V. (1996). Comparative gonopod morphology of the European spider crabs of the genus *Maja* Lamarck 1801 (Crustacea: Decapoda: Brachyura: Majidae). *Senckenbergiana biologica*, 75, 143-158.

Ng PKL, Chuang CTN. (1996). The Hymenosomatidae (Crustacea: Decapoda: Brachyura) of southeast Asia, with notes on other species. *The Raffles Bulletin of Zoology*, supplement no 3, 1-82.

Ng PKL, Guinot D, Davie PJF. (2008). Systema Brachyurorum: Part I. An annotated checklist of extant brachyuran crabs of the world. *Raffles Bulletin of Zoology*, 17, 1-286.

Ocampo EH, Luppi TA, Spivak ED, Klaus S. (2018). The ontogeny of the female reproductive system in the parasitic castrator pea crab *Calyptraeotheres garthi*: Implications for its mating system. *Journal of Morphology*, 279, 531-544.

Oh SJ, Hankin DG. (2004). The sperm plug is a reliable indicator of mating success in female Dungeness crabs, *Cancer magister*. *Journal of Crustacean Biology*, 24, 314-326.

Orensanz JM, Parma AM, Armstrong DA, Armstrong J, Wardrup P. (1995). The breeding ecology of *Cancer gracilis* (Crustacea: Decapoda: Cancridae) and the mating systems of cancrid crabs. *Journal of Zoology*, 235, 411-437.

Pardo LM, Riveros M, Fuentes JP, López-Greco LS. (2013). Functional morphology of the seminal receptacle in the crab *Metacarcinus edwardsii*. *Invertebrate Biology*, 132, 386-393.

Pardo LM, Ceroni C, Riveros MP, Ernst B, Pino J. (2017). Morphology of seminal receptacle of the harvested golden crab *Chaceon chilensis* and its implication in the fertilization process. *Invertebrate Biology*, 136, 199-206.

Poore GCB. (2010). *Elamenopsis guinotae*, a new spider crab from Bass Strait, Australia (Brachyura, Hymenosomatidae). In: Davie PJF, Ng PKL, Richer de Forges B, editors. Studies on Brachyura: a Homage to Danièle Guinot Crustaceana Monographs. Leiden: Koninklijke Brill NV. p 261-268.

Porter ML, Pérez-Losada M, Crandall KA. (2005). Model-based multi-locus estimation of decapod phylogeny and divergence times. *Molecular Phylogenetics and Evolution*, 37, 355-369.

Rathbun MJ. (1925). The spider crabs of America. *Bulletin of the United States National Museum*, 129, 1-613.

Relini M, Orsi L, Puccio V, Azzurro E. (2000). The exotic crab *Percnon gibbesi* (H. Milne Edwards, 1853)(Decapoda, Grapsidae) in the Central Mediterranean. *Scientia Marina*, 64, 337-340.

Richer de Forges B, Jamieson BGM, Guinot D, Tudge CC. (1997). Ultrastructure of the spermatozoa of Hymenosomatidae (Crustacea: Brachyura) and the relationships of the family. *Marine Biology*, 130, 233-242.

Rorandelli R, Paoli F, Cannicci S, Mercati D, Giusti F. (2008). Characteristics and fate of the spermatozoa of *Inachus phalangium* (Decapoda, Majidae): description of novel sperm structures and evidence for an additional mechanism of sperm competition in Brachyura. *Journal of Morphology*, 269, 259-271.

Rotllant G, González-Gurriarán E, Fernández L, Benhalima K, Ribes E. (2007). Ovarian maturation of the multi-spawning spider crab *Maja brachydactyla* (Decapoda: Majidae) with special reference to yolk formation. *Marine Biology*, 152, 383-394.

Ryan EP. (1965). A study of the reproductive biology of the haole crab, *Portunus sanguinolentus* (Herbst)(Brachyura: Portunidae) [Dissertation]: University of Haway, Honolulu. 1-209 p.

Sainte-Marie B, Lovrich GA. (1994). Delivery and storage of sperm at first mating of female *Chionoecetes opilio* (Brachyura: Majidae) in relation to size and morphometric maturity of male parent. *Journal of Crustacean Biology*, 14, 508-521.

Sainte-Marie G, Sainte-Marie B. (1998). Morphology of the spermatheca, oviduct, intermediate chamber, and vagina of the adult snow crab (*Chionoecetes opilio*). *Canadian Journal of Zoology/Revue Canadienne de Zoologie*, 76, 1589-1604.

Sainte-Marie G, Sainte-Marie B, Sévigny J-M. (2000). Ejaculate-storage patterns and the site of fertilization in female snow crabs (*Chionoecetes opilio*; Brachyura, Majidae). *Canadian Journal of Zoology/Revue Canadienne de Zoologie*, 78, 1902-1917.

Sal Moyano MP, Gavio MA. (2012). Comparison of mating behavior and copulation in male morphotypes of the spider crab *Libinia spinosa* (Brachyura: Majoidea: Epialtidae). *Journal of Crustacean Biology*, 32, 31-38.

Sal Moyano MP, Gavio MA, Cuartas EI. (2010). Morphology and function of the reproductive tract of the spider crab *Libinia spinosa* (Crustacea, Brachyura, Majoidea): pattern of sperm storage. *Helgoland marine research*, 64, 213-221.

Sal Moyano MP, Gavio MA, Cuartas EI. (2011). Copulatory system of the spider crab *Libinia spinosa* (Crustacea: Brachyura: Majoidea). *Journal of the Marine Biological Association of the United Kingdom*, 91, 1617-1625.

Sant'Anna BS, Pinheiro MAA, Mataqueiro M, Zara FJ. (2007). Spermathecae of the mangrove crab *Ucides cordatus*: a histological and histochemical view. *Journal of the Marine Biological Association of the United Kingdom*, 87, 903-912.

Scholtz G. (2014). Evolution of crabs – history and deconstruction of a prime example of convergence. *Contributions to Zoology*, 83, 87-105.

Scholtz G, Richter S. (1995). Phylogenetic systematics of the reptantian Decapoda (Crustacea, Malacostraca). *Zoological Journal of the Linnean Society*, 113, 289-358.

Scholtz G, McLay CL. (2009). Is the Brachyura Podotremata a monophyletic group? In: Martin JW, Crandall KA, Felder DL, editors. Crustacean Issues 18: Decapod Crustacean Phylogenetics. Boca Raton, Florida: Taylor & Francis/CRC Press. p 417-435.

Schubart CD, Reuschel S. (2009). A proposal for a new classification of Portunoidea and Cancroidea (Brachyura: Heterotremata) based on two independent molecular phylogenies. In: Martin JW, Crandall KA, Felder DL, editors. Crustacean Issues 18: Decapod Crustacean Phylogenetics. Boca Raton, Florida: Taylor & Francis/CRC Press. p 533-549.

Schubart CD, Cuesta JA. (2010). Phylogenetic relationships of the Plagusiidae Dana, 1851 (Brachyura), with description of a new genus and recognition of Percnidae Stevcic, 2005, as an independent family. Studies on Brachyura: a Homage to Danièle Guinot. Leiden: Koninklijke Brill NV. p 279-300.

Schubart CD, Cuesta JA, Diesel R, Felder DL. (2000). Molecular phylogeny, taxonomy, and evolution of nonmarine lineages within the American grapsoid crabs (Crustacea: Brachyura). *Molecular Phylogenetics and Evolution*, 15, 179-190.

Schubart CD, Cuesta JA, Felder DL. (2002). Glyptograpsidae, a new brachyuran family from Central America: larval and adult morphology, and a molecular phylogeny of the Grapsoidea. *Journal of Crustacean Biology*, 22, 28-44.

Schubart CD, Cannicci S, Vannini M, Fratini S. (2006). Molecular phylogeny of grapsoid crabs (Decapoda, Brachyura) and allies based on two mitochondrial genes and a proposal for refraining from current superfamily classification. *Journal of Zoological Systematics and Evolutionary Research*, 44, 193-199.

Secretan S. (1998). The sella turcica of crabs and the endophragmal system of decapods. *Journal of Natural History*, 32, 1753-1767.

Shen CJ. 1935. An investigation of the post-larval development of the shore-crab *Carcinus maenas*, with special reference to the external secondary sexual characters. *Proceedings of the Zoological Society of London*, 105, 1-34.

Shen H, Braband A, Scholtz G. (2013). Mitogenomic analysis of decapod crustacean phylogeny corroborates traditional views on their relationships. *Molecular Phylogenetics and Evolution*, 66, 776-789.

Sin YW, Lai JCY, Ng PKL, Chu KH. (2009). Phylogeny of Dorippoidea (Crustacea: Decapoda: Brachyura) inferred from three mitochondrial genes. *Invertebrate Systematics*, 23, 223-230.

Spalding JF. (1942). The nature and formation of the spermatophore and sperm plug in *Carcinus maenas*. *Quarterly Journal of Microscopical Science*, 2, 399-422.

Spears T, Abele LG, Kim W. (1992). The Monophyly of Brachyuran Crabs: A Phylogenetic Study Based on 18s rRNA *Systematic Biology*, 41, 446-461.

Stephensen K. (1946). The Brachyura of the Iranian Gulf. With an appendix: the male pleopoda of the Brachyura. In: Jessen K, Spärck R, editors. Danish scientific investigations in Iran. p 57-237.

Števčić Z. (2005). The reclassification of brachyuran crabs (Crustacea: Decapoda: Brachyura). *Natura Croatica*, 14, 1-159.

Swartz RC. (1978). Reproductive and molt cycles in the xanthid crab, *Neopanope Sayi* (Smith, 1869). *Crustaceana*, 34, 15-32.

Talbot P, Demers D. (1993). Tegumental glands of Crustacea. In: Horst MN, Freeman JA, editors. The crustacean integument: morphology and biochemistry. Boca Raton: CRC Press. p 151-191.

Tang B-P, Xin Z-Z, Liu Y, Zhang D-Z, Wang Z-F, Zhang H-B, Chai X-Y, Zhou C-L, Liu Q-N. (2017). The complete mitochondrial genome of *Sesarmops sinensis* reveals gene rearrangements and phylogenetic relationships in Brachyura. *PloS one*, 12, e0179800.

Tavares M, Secretan S. (1993). La notion de thelycum et de spermathèque chez les Crustacés Décapodes. *Comptes Rendus de l'Académie des Sciences - Series III*, 316, 133-138.

Teske PR, Papadopoulos I, Zardi GI, McQuaid CD, Edkins MT, Griffiths CL, Barker NP. (2007). Implications of life history for genetic structure and migration rates of southern African coastal invertebrates: planktonic, abbreviated and direct development. *Marine Biology*, 152, 697-711.

Teske PR, McLay CL, Sandoval-Castillo J, Papadopoulos I, Newman BK, Griffiths CL, McQuaid CD, Barker NP, Borgonie G, Beheregaray LB. (2009). Tri-locus sequence data reject a "Gondwanan origin hypothesis" for the African/South Pacific crab genus *Hymenosoma*. *Molecular Phylogenetics and Evolution*, 53, 23-33.

Thompson GA, McLay CL. (2005). Mating behaviour of *Heterozius rotundifrons* (Crustacea: Brachyura: Belliidae): is it a hard or soft shell mater? *Marine and Freshwater Research*, 56, 1107-1116.

Tsang LM, Schubart CD, Ahyong ST, Lai JCY, Au EYC, Chan T-Y, Ng PKL, Chu KH. (2014). Evolutionary history of true crabs (Crustacea: Decapoda: Brachyura) and the origin of freshwater crabs. *Molecular biology and evolution*, 31, 1173-1187.

Tsuchida S, Fujikura K. (2000). Heterochely, relative growth, and gonopod morphology in the bythograeid crab, *Austinograea williamsi* (Decapoda, Brachyura). *Journal of Crustacean Biology*, 20, 407-414.

Vallina M, Sal Moyano MP, Cuartas EI, Gavio MA. (2014). Reproductive system and size maturity of the paddle crab *Ovalipes trimaculatus* (Brachyura: Portunidae) along the Argentine coast. *Journal of Crustacean Biology*, 34, 357-366.

van den Brink AM, McLay CL. (2009). Use of the sterile male technique to investigate sperm competition, storage, and use in a pill box crab, *Halicarcinus cookii* (Brachyura: Hymenosomatidae). *Journal of Crustacean Biology*, 29, 62-69.

Vehof J, van der Meij SET, Türkay M, Becker C. (2016). Female reproductive morphology of coral-inhabiting gall crabs (Crustacea: Decapoda: Brachyura: Cryptochiridae). *Acta Zoologica (Stockholm)*, 97, 117-126.

Vehof J, Scholtz G, Becker C. (2017). Morphology of the female reproductive system of three dorippid crabs (Crustacea: Decapoda: Brachyura: Dorippidae) and the role of accessory cuticle structures associated with seminal receptacles. *Invertebrate Biology*, 136, 271-289.

Vehof J, Scholtz G, Becker C. (2018). *Paradorippe granulata* – A crab with external fertilization and a novel type of sperm storage organ challenges prevalent ideas on the evolution of reproduction in Eubrachyura (Crustacea: Brachyura: Dorippidae). *Arthropod structure & development*, 47, 82-90.

Vinuesa JH, Ferrari L. (2008). Reproduction of *Halicarcinus planatus* (crustacea, decapoda, hymenosomatidae) in the Deseado River estuary, southwestern Atlantic Ocean. *Marine Biology*, 154, 345-351.

Von Sternberg R, Cumberlidge N. (2001). On the heterotreme-thoracotreme distinction in the Eubrachyura de Saint Laurent, 1980 (Decapoda, Brachyura). *Crustaceana*, 74, 321-338.

Von Sternberg R, Cumberlidge N, Rodriguez G. (1999). On the marine sister groups of the freshwater crabs (Crustacea: Decapoda: Brachyura). *Journal of Zoological Systematics and Evolutionary Research*, 37, 19-38.

Walker K. (1969). The ecology and distribution of *Halicarcinus lacustris* (Brachyura: Hymeno-somatidae) in Australian inland waters. *Marine and Freshwater Research*, 20, 163-174.

Yokes B, Galil BS. (2006). Touchdown-first record of *Percnon gibbesi* (H. Milne Edwards, 1853)(Crustacea: Decapoda: Grapsidae) from the Levantine coast. *Aquatic Invasions*, 1, 130-132.

Yuhui X, Lijun Z, Yue H, Xiaoqi W, Chen Z, Huilun Z, Ruoran W, Da P, Hongying S. (2017). Complete mitochondrial genomes from two species of Chinese freshwater crabs of the genus *Sinopotamon* recovered using next-generation sequencing reveal a novel gene order (Brachyura, Potamidae). *ZooKeys*, 705, 41-60.

Zara FJ, Toyama MH, Caetano FH, López-Greco LS. (2012). Spermatogenesis, spermatophore, and seminal fluid production in the adult blue crab *Callinestes danae* (Portunidae). *Journal of Crustacean Biology*, 32, 249-262.

Zara FJ, Pereira GRR, Sant'Anna BS. (2014). Morphological changes in the seminal receptacle during ovarian development in the speckled swimming crab *Arenaeus cribrarius*. *The Biological Bulletin*, 227, 19-32.

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